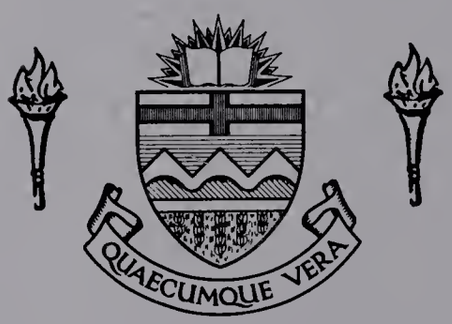

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METABOLIC AND CARDIOVASCULAR ADAPTATIONS IN THE WESTERN CHIPMUNKS, GENUS *EUTAMIAS*

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© DOUGLAS L. JONES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Metabolic and cardiovascular adaptations in the western chipmunks, genus Eutamias," submitted by Douglas L. Jones, in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

The metabolic and cardiovascular adaptations were studied in 2 species (5 subspecies) of Eutamias.

Estimated basal metabolic rates (EBMR) within the thermal neutral zone (TNZ) were 1.78 (E.m.bo.), 1.64 (E.m.or.), 1.50 (E.m.op.), and 1.69 ccO2/g-h (E.a.lut.) respectively, and 839, 752, 689, and 828 ccO2/kg$^{0.75}$-h respectively. There were no significant differences between "light" and "dark" EBMR within each subspecies. However, all subspecies had EBMR's which were significantly higher than weight predicted values. EBMR's of the boreal subspecies (E.m.bo. and E.a.lut.) were signifntly higher than those of the alpine subspecies (E.m.or. and E.m.op.).

Minimum thermal conductances (TC) were 0.1138, 0.1140, 0.1126, and 0.1126 ccO2/g-h-C respectively; and 54.4, 54.0, 50.4, and 52.1 ccO2/kg$^{0.75}$-h-C respectively. These values are significantly lower than their weight predicted values. It is postulated that the depression of TC could be accounted for by the relatively long fur and postural adjustments. The TC's of alpine subspecies when adjusted to body size were lower than that of the boreal subspecies, suggesting that the alpine subspecies were better adapted for cold exposure.

Estimated basal heart rates (EBHR) of the four subspecies were 49 to 55 percent of weight specific values.

The calculated oxygen pulses (OP) were 5.49, 4.50, 4.48, and 5.56 $x10^{-3}$ ccO2/beat respectively, which were 2 to 2.4 times their weight specific values.

The increased OP was speculated to be due to either an increase in stroke volume (SV) and/or A-V O2 difference. Such increase would have compensated for the depression of EBHR in meeting the demand for an elevated EBMR. From calculated values of SV and indirect evidence from heart weight, it was concluded that most of the modifications of OP come from the factors contributing to an increased A-V O2 difference.

Blood oxygen capacity was calculated to be 20.6, 19.7, 18.9, and 22.4 ccO2/100 cc blood respectively. The measured A-V O2 differences in anaesthetized animals averaged 5.6 (E.m.or.), 7.8 (E.m.op.), and 10.4 vol % (E.a.lut.) respectively, with differences as high as 13.4 vol % observed in E.a.lut.. It is speculated that with greater oxygen demands, A-V O2 differences may increase further for better oxygen extraction. This, coupled with the ability to rapidly increase the heart rate through neural processes, would provide the chipmunks with great cardiovascular reserves for aerobic metabolism.

Chipmunks were found to exhibit circadian rhythms in heart rate (HR), body temperature (Tb), and oxygen consumption. They were also reported to exhibit adaptive behavioral patterns, such as the reduction of interspecific

aggression by dominance heirarchy. The sum of these observations are considered to be advantageous to the survival of chipmunks as well as enabling the chipmunks to radiate to their present distribution by successfully outcompeting the existing species.

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INTRODUCTION

Chipmunks appear to be capable of adapting to very diverse habitats: from desert (Brown, 1973; Brown and Lieberman, 1973; Hall and Kelson, 1959; Heller, 1971; Heller and Gates, 1971), to high alpine and northern areas (Armstrong, 1972; Hall and Kelson, 1959; Heller, 1971; Heller and Gates, 1971; Smith, 1974). The Western Chipmunks of the genus Eutamias, which evolved from the Tamias tribe in the Miocene (Black, 1963) were thought to have invaded habitats previously occupied by other rodents, many of which were in direct competition for food and space with the chipmunks (Armstrong, 1972). The present day wide distribution of the chipmunks (Burt and Grossenherder, 1964; Hall and Kelson, 1959) suggests that special physiological and/or behavioral adaptations might have evolved which enabled these animals to outcompete existing species and to invade new territories.

Literature concerning taxonomy and morphology (Black, 1963; Bryant, 1945; Burt, 1960; Howell, 1929, 1938; Nadler, 1964; White, 1953a, 1953b, 1953c, 1953d), ecology and seasonal variations of certain parameters such as body weight, organ weight, hibernation and behavioral patterns, (Armstrong, 1972; Jameson, 1964; Jameson and Mead, 1964; Merideth, personal communication; Sheppard, 1965, 1968, 1971; Smith, 1974) of western chipmunks are relatively abundant. On the other hand, only a handful of studies are

devoted to measurements of physiological parameters (Heller, 1971; Heller and Gates, 1971; Heller and Poulson, 1970; Wunder, 1970a, 1970b). These studies provide basic reference values for energy metabolism of chipmunks either of a seasonal character or of individual species' total energy requirements. The implications derived from these studies have been vast; a notable example has been the use of such findings in correlation with the altitudinal zonation of species in the Sierras (Heller and Gates, 1971).

In the Eastern chipmunk, Tamias striatus, Wang and Hudson (1971) observed a suppression of basal heart rate (BHR) to be about 70% of its weight specific value as predicted by the empirical formulae of Spector (1956) and Stahl (1967). The equation is:

$$HR = 241\ W^{-0.25}$$

Where HR is the heart rate in beats/min,

and W is the animal weight in kilograms.

The suppression appeared to be under strong neural inhibition of the vagus nerves. The basal metabolic rate (BMR) of the animal, on the other hand, was essentially the same as its weight specific values (Wang and Hudson, 1971). Since the correlation between metabolism and cardiovascular function is expressed in the following equations:

$$MR = (CO) \times (A\text{-}V\ O_2 \text{ difference})$$

and

$$CO = (SV) \times (HR)$$

and, therefore,

MR = (HR) x (SV) x (A-V O2 difference)

where MR is metabolic rate in ccO2/min,

HR is heart rate in beats/min,

CO is cardiac output in cc blood/min,

SV is stroke volume in cc blood/beat,

and A-V O2 difference is arterial and venous blood oxygen content difference in ccO2/100 cc blood,

the fact that T. striatus is capable of maintaining a normal BMR at a depressed BHR (Wang and Hudson, 1971) suggests that sufficient compensation must have been made by the animal through the increase of either its SV and/or its A-V O2 difference. However, direct experimental evidence to support that such is the case is still lacking at the present.

It is speculated that cardiovascular modifications similar to those found in Tamias may also exist in Eutamias. Observations supporting such a hypothesis are: (1) the ancestral relationship with Tamias; (2) the presence of E. minimus at high altitudes (up to 4700 m, Hall and Kelson, 1959), where the relatively low oxygen partial pressure (60% of sea level value, Bard, 1961) may have selected for greater efficiency in procurement and/or transport of oxygen from the environment to the cells than that required at low altitudes; (3) the typical quick dash - still - quick dash pattern of activity bursts in Eutamias may require cardiovascular function capable of dynamic changes in response to demand of oxygen uptake, i.e. good cardiovascular reserve; and (4) preliminary studies

indicated that several species of Eutamias showed depression of BHR at normal body temperature (Tb) (Wang, personal communication). Although the BMR was not measured, the normal Tb suggests it was probably normal.

The objectives of this study therefore, were: (1) to discern the metabolic performance of Eutamias from different habitats; (2) to discern any modifications in cardiovascular parameters related to metabolism; and (3) to correlate metabolic and cardiovascular modifications as strategies used by the animals as adaptations to their environments.

MATERIALS AND METHODS

One of the major difficulties in studying physiological parameters in Eutamias is the relatively small size of the animals, ranging from 30 to 100 g (Armstrong, 1972; Hall and Kelson, 1959). Modifications of existing techniques were therefore necessary in order to obtain standard physiological measurements. Detailed descriptions of many such modifications are included in this thesis because of their potential application to other similar sized animals.

ANIMALS

The two species and five subspecies of Eutamias used in this study were collected by three individuals. In all cases, the animals were trapped using Sherman live traps, either 15.2 x 6.4 x 5.6 cm or 22.9 x 8.9 x 7.6 cm in dimension.

Twenty five Eutamias minimus borealis (Allen) (hereafter referred to as E.m.bo.) were collected in the boreal regions from three locations: the Heart Lake area of the Northwest Territories in 1970, 1971 (see Smith, 1974 for descriptions of the area and climatic conditions), the Jasper National Park area of Alberta in 1973 (D. Merideth, personal communication), and the Rocky Mountain House region of Alberta in 1972.

Sixty two specimens of Eutamias minimus oreocetes

(Merriam) (E.m.or.) which inhabit the alpine Krumholtz and tallus regions of mountain slopes were trapped by the author and D. Merideth. These animals were trapped in the vicinity of Gibraltar Mountain approximately 48 km west of Turner Valley, Alberta; the Sheep River summit approximately 66 km west of Turner Valley; the head waters of the Elbow River and Goat Valley approximately 80 km west of Turner Valley; and the surrounding regions in 1972 and 1973.

Fifty four specimens of Eutamias amoenus luteiventris (Allen) (E.a.lut.) were trapped by the author and by D. Merideth in 1972 and 1973. These animals basically were parapatric with the E.m.or., however, they inhabited the boreal portions and bordered the clearings (Merideth, 1974; Sheppard, 1971, 1972).

Thirteen Eutamias amoenus ludibundis (Hollister) (E.a.lud.) were collected in the Jasper National Park in 1973 by D. Merideth. These animals were allopatric to E.m.or.. It is interesting to note here that the minimus species is in the alpine area and the amoenus species in the boreal in southern Alberta, while the reverse is true in northern Alberta with amoenus in the alpine and minimus in the boreal.

Due to the relatively small sample size of E.a.lud., only limited measurements were made on these animals. The data are included for information only, and no statistical comparisons were made with other species.

In the summer of 1973, the author collected 33 Eutamias minimus operarius (Merriam) (E.m.op.) near Aspen, Colorado. Animals were collected from 7 different sites in a 400 square km area surrounding Ashcroft at altitudes from 1,970 m to 3,640 m. These sites occurred in the life zones best described by the Canadian zone and the Hudsonian zone, as described by Armstrong (1972). The identification of these animals followed Armstrong (1972) and Lechleitner (1969). The animals were transported to the University of Alberta by car.

All the animals were kept individually in plastic cages measuring 50.8 x 40.6 x 21.6 cm at an ambient temperature (Ta) of 16 to 18C under 12L:12D photoperiod in the University of Alberta Laboratory Animal Services. Food (Vitamite cubes) and water were provided ad. libitum, supplemented with sunflower seeds. Bedding provided was wood shavings and a small quantity of hay. Animals were left to acclimate under these conditions for a minimum of 2 weeks prior to measurements of physiological parameters.

In October, 1972, four E.a.lut., three E.m.bo., and two E.m.or. were placed into a walk-in environmental chamber approximately 2.5 x 3.5 m x 2 m high inside dimensions for observation of hibernation episodes. Initially, the Ta and photoperiod regime inside the chamber followed the local changes (between October and December 22, 1972). However, due to failure of machinery, this scheme could not be

continued after December 22, 1972. Subsequently, the animals were under a 12L:12D photoperiod and constant Ta of 10C (December 22, 1972 to March 29, 1973). "Light" in this text will mean the light period of the holding quarters only, and "dark" will mean the dark period of the holding quarters only.

OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION

Oxygen consumption measurements were obtained by an open circuit system utilizing a Beckman G-2 paramagnetic oxygen analyzer with the output from the analyzer recorded by a Honeywell Electronic 15 strip chart recorder. The accuracy of these measurements was ±1.0% of full scale. In many cases, the output from the oxygen analyzer was synchronously computed by a Texas Instruments (T.I.) computer model #980A.

Air was supplied from the compressed air line in the laboratory. It was first decompressed to 5 psi by a low pressure regulator (Matheson model #70), and then dried by being passed through a tube which contained Drierite ($CaSO_4$, Fisher Scientific Co.). Flow rate to the animals was metered by individually calibrated flow meters (Matheson models 602 and 603) before it reached the animal chamber. The animal chamber was held within a temperature control box which maintained Ta inside the animal chamber to ±0.5C of the desired value.

The exhaust from the animal chamber was split into two streams, one for oxygen analysis, and one for carbon dioxide (CO_2) analysis. The stream to the oxygen analyzer was passed through a tube containing Ascarite and Drierite for CO_2 and water removal. The stream to the CO_2 analyzer passed through a tube containing Drierite only. The CO_2 production was measured by a Beckman Infrared Analyzer (Model #864). The output of the CO_2 analyzer was recorded on a Fisher recorder (model #13-939-10, Fisher Scientific Co.). Often, the output was simultaneously recorded on the T.I. computer, which also computed the instantaneous and accumulated respiratory quotient (RQ).

Calculation of oxygen consumption was made using the method of Depocas and Hart (1957), with the second assumption of Hill (1972) implicit.

Measurements of total oxygen consumed per hour were obtained by cutting out the section of chart paper along the recording pen tracing above the base line, equivalent to one hour of measurement, and weighing the paper to the nearest 0.5 mg. The absolute value was then determined by comparing this weight with that of a standard area of chart paper equivalent to a unit of oxygen consumption value.

Calculation of CO_2 production was based on the same principle as that for the oxygen consumption. This principle calls for the correction of fractional concentration of O_2

or CO2 since the volume of exhaust gas passing to the analyzer may or may not be equal to the gas volume flowing into the animal chamber. The two are equal only if the RQ of the animal is equal to 1. In order to obtain accurate measurement of CO2 production irrespective of RQ values, the following equation was derived for calculation.

$$\dot{V}CO2 = (FECO2(\dot{V}I-\dot{V}O2)-(\dot{V}I \times FICO2))/(1-FECO2)$$

Where:

$\dot{V}CO2$ = volume of CO2 produced by the animal corrected to STP

$\dot{V}I$ = volume of dry inlet air flowing to the animal per unit time corrected to STP

FICO2 = fractional concentration of CO2 in the inlet air

$\dot{V}O2$ = volume of oxygen consumed by the animal corrected to STP per unit time

FECO2 = fractional concentration of CO2 in dry outlet air as monitored by the CO2 analyzer

Two important factors in this equation which may often be neglected are the subtraction of the instantaneous volume of oxygen consumed by the animal, and the subtraction of the CO2 in the inlet air.

The respiration chambers utilized in this work were of four basic designs. The first design was used for the majority of the measurements which were to give values of

metabolism and other parameters on unanaesthetized non-active animals. The chambers were constructed from paint tins which had volumes of 1136 cc. These cans had either three or four ports made out of 6 mm copper tubing. The inlet port was sealed at the bottom and had holes drilled into the portion of the tube which opposed the nearest wall. This direction of air flow was thought to give the greatest amount of turbulence, and increase the mixing of the air in the chamber, thus reducing the total flush time. The exhaust port was short and straight, entering the chamber only about 1 cm. The third port was also sealed on the bottom. This tube was utilized to hold the Ta thermocouple. The sealed portion of the inlet and Ta tubes were approximately 1 cm from the bottom of the can in a position which would have approximated the position of the animal once the bedding was in place. The fourth tubing was present only in some of the chambers. This tube was placed in the center of the lid. The inner end was as near as possible flush with the inside surface of the lid. Once a tygon tube was placed over the outer end of the tube and the electrodes from the animal passed through the lumen of both tubes, a clamp was tightened on the tygon tubing, sealing the chamber and making it air tight. (The methods for electrode attachment will be discussed later.)

The flow rates utilized in this type of chamber were maintained between 364 and 1500 cc/min (STP). The actual flushing time to change an equilibrated oxygen content of

18.93 to 20.93% at 364 cc/min was found to be 8 minutes. Since this was the lowest flow rate used in this study, and also without the animal inside the chamber, (which would reduce the total volume for flushing and further increase the turbulence), this 8 minute value was considered to be the maximum time lag in the measuring system. At higher flow rates, this time would be expected to approach less than 5 minutes.

In all experiments, the animals were left for a minimum of 3 hours at any one temperature. Readings were taken on minimum stabilized readings which usually had remained stable for 10 minutes or longer to ensure they were equilibrated values. During most runs, the difference of oxygen content between inlet and exhaust air did not exceed 1%.

Initially, the animals were deprived of food for a period of 24 hours to ensure a postabsorbative state, prior to metabolic measurements. However, after the consecutive loss of 3 animals, this practice was abandoned. Instead, nonfasted animals were used, but no readings were taken in the initial 3 hours of each run.

The second and third types of chamber were more restrictive on the movements of animals. Since these chambers were only used for analysis on anaesthetized animals, and flow rates were held at approximately 600 cc/min with Ta in the thermal neutral zone (TNZ), the

restriction was not considered critical. The greater flow rate also gave very rapid dynamic responses.

Chamber type 2 was constructed out of plexiglass. The inner dimensions of this chamber were 10.2 x 10.2 x 15.2 cm long. One end was equipped with a "T" baffel air inlet made of 6 mm copper tubing. The other end had a 7.2 cm (diameter) hole for a size 14 rubber stopper. The stopper was equipped with two 6 mm copper tubings. One tube was sealed and acted as the Ta probe holder. The other tube was for air exhaust. The top of the box had a hole 1.4 cm in diameter for a size 0 1-hole rubber stopper. Through the hole, the implanted electrodes and cannulae from the animal could be passed. During measurements, the possible air leak from this hole was eliminated by filling the space with high vacuum silicon grease. This allowed for simultaneous measurements of oxygen consumption, carbon dioxide production, instantaneous RQ, heart rate and arterial and venous blood gas analysis (A-V O2 difference). Cardiac output (CO) and stroke volume (SV) were calculated from appropriate values obtained in these measurements (details to follow).

The type three chamber was constructed out of a 150 mm Nalgene dessicator jar (Canadian Laboratory Supplies). The total volume of the jar was 1450 ml. A perforated metal plate 2 cm from the bottom supported the animal. Three holes were drilled into the sides of the jar. Into two of these holes, the barrels of two 1 cc disposable syringes were

glued with epoxy resin. These served as air inlet and exhaust. Into the third hole, the protective plastic casing from a disposable hypodermic needle was glued with the opening towards the exterior. This was used to house the Ta thermocouple.

A hole was drilled in the center of the lid and a 1 cc disposable syringe barrel was glued to it. Through this tube the electrodes and cannulae were passed during analysis. This tube could also be plugged with high vacuum silicon grease to give an air tight seal. The sides of the jar were held tight with the use of 6 metal spring paper holders (Foldback #1412 paper clip - Myers, Birmingham). This chamber was found superior to type 2 due to its ease of opening and thus, access to the animal. Therefore, it replaced the use of chamber type 2 very early in the study.

The fourth type chamber was a treadmill respirometer which was a modified design from Wunder (1972b). It was powered by a Zero-Max motor (JK-1) which was capable of 0 to 400 rpm at 25 ftlbs torque. It was constructed by the University of Alberta Technical Services Branch. The inside dimensions were 35.6 x 17 x 16.5 cm deep. With the insertion of removable wooden blocks to decrease the dead space of the chamber, the flushing time for 99% equilibrium from 18.93 to 20.93% oxygen was 7 minutes at flow rates of 1500 cc/min. The flushing time to reach 90% equilibration was reduced to less than 2 min at a flow rate of 2000 cc/min (STP). Four 6

mm copper tubings were utilized for air inlet and exhaust, Ta thermocouple insertion and an electrode outlet port.

HEART RATE

Two methods were used for measuring heart rate. The initial method was the use of direct electrode implants. A saddle pack was constructed from one half of a miniature 14 prong integrated circuit plug (IC), (Barnes P-041 Series, Smalley's Electronics), and a piece of plastic from the barrel of a 25 cc disposable syringe. It measured 15 mm long and 6 mm wide. A rectangular portion was removed from the center to house the IC plug. The plug was glued to the plastic with epoxy resin, with the copper pins opposing the outward curve of the plastic. The curvature of the plastic was similar to the nape of the neck of the animal. Holes were drilled into the four corners of the saddle for attachment to the skin by opthalmic silk sutures. The 3 electrocardiogram (EKG) electrodes were prepared from 40 gauge constantan wire (Sigmund Cohn Corp., Mt. Vernon, N.Y.). One was implanted in each shoulder and one in the rump. Two copper-constantan thermocouples were made out of 40 gauge wires also. The first thermocouple (Tb1) was implanted in the abdominal cavity immediately posterior to the kidneys and ventral to the spinal column. Autopsy on 3 animals revealed that the thermal sensitive junction was indeed in this position, and was immediately posterior and proximal to the liver. Such a placement would give a very

accurate reading of the deep Tb. The second thermocouple (Tb2) was placed through the muscle in the back of the neck.

Prior to implantation, the animal was anaesthetized with 50 mg/kg "ip" Nembutal (sodium pentabarbital), which was supplemented when necessary during implantation with anaesthetic grade ether.

All wires were brought subcutaneously to the base of the neck. The wires were exteriorized, and the leads directly soldered onto the appropriate pins of the IC plug. The saddle was then sutured onto the back, and the animals were returned to their cages.

To reduce the restriction of movement of the animals during measurements due to hard wires, a special electrical swivel connector was constructed which contained 7 concentric mercury baths. In a piece of 1.9 x 8 cm in diameter plexiglass, a center hole 1 cm in diameter was drilled. Subsequently, 7 concentric circles were drilled 2 mm wide and 2 mm apart, starting 2 cm from the center hole to a depth of 1.5 cm. Into the bottom of each trough was screwed a 4 mm chrome plated screw which connected appropriate animal electrodes, through the mercury when the troughs were filled, to appropriate recording leads connected to the chrome plated screw. A female 7 prong matching IC plug with extension leads was attached to the male IC of the back-pack prior to each measurement. The leads were passed through the lumen of a 3.2 mm stainless

steel tubing approximately 5 cm long. This tube sat in a "T" teflon bushing which was inserted into the center hole of the plexiglass plate. The tube was prevented from slipping through the bushing by a teflon washer glued to it by epoxy resin.

Once the troughs were filled with mercury, the lead wires from the animal were bent, first horizontally and then downward, so that the scrapped end portion of lead was in contact with the mercury. Thus, when the animal moved around, the leads would rotate without impeding the animal's motion, yet maintaining electrical contact at the same time. It was soon discovered, however, that if the animal stood on its hind legs, the leads were elevated far enough to lose contact with the mercury. To prevent this, a piece of plastic was glued onto the teflon washer in the horizontal plane with notches in positions above the concentric troughs. The lead wires could then be twisted around the plastic cross beam, and then bent into the mercury. This special electrical connector could either be held within the chamber by connecting nuts to the lid, or supported on the top of 6.3 mm wire mesh covering the chamber if oxygen consumption was not to be measured. This arrangement allowed the animal to move around inside the can. However, the 40 gauge wire was not very resilient, and implanted leads rarely lasted more than 4 or 5 days.

The second method of heart rate measurement was by

biotelemetry. Because of the small size of the chipmunks studied (about 50 g), it was not possible to use any of the commercially available EKG transmitters without imposing severe pathological and psychological stresses. It was therefore necessary to develop an EKG transmitter specifically for the present study. The details of the transmitters are described under the section "Transmitters".

Field measurements of heart rate versus activity and Ta were obtained from an implanted animal maintained in pen B described by MacArthur and Wang (1974). The pen was situated in an aspen grove 6.5 meters from recording equipment at Gorge Creek Biological Station, approximately 30 km west of Turner Valley, Alberta (MacArthur, 1973). The pen contained a rock pile at one end, and shavings and some hay were provided for bedding.

Transmitted EKG signals were recieved by a Sony CF-300 AM/FM Radio-Cassette-Corder (General Distributor, Calgary, Alberta). Signals were either recorded directly by a Grass polygraph (model 7, Grass Instruments, Quincy, Massachusetts), or a physiograph (Narco-Biosystems, Toronto, Ontario) by attaching recording leads to the earphone monitor of the Sony recorder, or were taped on magnetic cassette tape and later transcribed by a polygraph or physiograph. The cassette taping process was not always successful due to the residual frequencies on old tapes from incomplete erasure, the interference of 64 hertz and its

harmonics when the Sony recorder was connected to 115 volt AC source, and the aberrant FM signals sometimes produced by nearby electrical equipment.

Periodical recordings were taken at one of three time intervals: 20 seconds every 5 minutes; 90 seconds every 30 minutes; or 150 seconds every 60 minutes. Usually, stabilized recordings were obtained within a 60 second interval. Heart rate was also recorded on the T.I. computer by feeding signals from the Sony recorder or oscillograph into it. The rate which was computed from 8 consecutive heart beat intervals was displayed on a TTI Teletype as beats/min. The computer also measured total accumulated heart beats for a given time interval.

Three lowest heart rates per run for each Ta between 0 and 25C during the "light" were used to compute least square linear regression for heart rate versus Ta.

TRANSMITTERS

The advent of telemetry technology has removed much of the movement restriction previously imposed on animals on which physiological measurements were desired. Haahn (1965) as quoted by Roy and Hart (1966) stated some of the prerequisites for a perfect telemetry package. These included:

> size - zero; range - infinite; power supply life - infinite; sensor and transducer pathological effects - none; degree of automation - complete, i.e. no

electronics background required for using the equipment.

A search of the literature (see Will and Patric, 1972 for a partial list of 455 sources), and a check of the commercially available packages (Buckle, 1973; Ives et al., 1973; Johannesson et al., 1973; Kurt and Kang, 1972; Lambrew et al., 1973; Studier and Howell, 1969; Zervanos and Hadley, 1973, to name only a few), revealed that there was not a transmitter which could come close to all these prerequisites.

For animals under 500 g, there have been the added problems of: producing a strong enough signal from a very small package so that short or medium range receiving is possible; and providing a reasonable battery life for the duration of the study. Most commercially available transmitters either had a one or two day battery life, or were only capable of transmitting from the gut of the subject to the surface of the subject. Anderka and Dyer (1967) described a 3.5 g transmitter, but the total expected life was only 30 hours. Roy and Hart (1966) and Hart and Roy (1966) described two transmitters which were capable of 2 mile transmission distances while the bird was in flight, but they were too large for the present study and the useful life spans of the transmitters were only 46 hours. Carson et al. (1971) modified the EEG transmitter circuit of Sperray et al. (1961), and achieved 4 week battery life in a 2 g transmitter which required a variety of receiving aerials to

achieve good reception in a package 1.2 x 2.0 cm. Reece et al. (1970) and Mitchell and Siegel (1973) have described EKG transmitters similar to type 3 of this study, but no information on range of transmission was included. Morhardt (personal communication) modified a circuit from MacKay (1970) and achieved transmission up to 10 feet.

In order to meet the requirements of the present study, it was decided that the transmitter described by Morhardt (personal communication) was the most suitable one. However, only very limited success was met when this transmitter was implanted. Further modifications of this transmitter were therefore necessary, so that heart rate in unrestrained chipmunks could be obtained.

The initial modifications (Fig. 1) involved the reduction in resistance values of R3, R4, and R5. This was necessary to supply a sufficient base bias to ensure that T2 was not turned off by the negative pulse coming from T1. This problem was accentuated by the increased current gain across T1 when the connections of E & F pickups allowed a DC current to flow if C2 was left out. L1 was a single turn coil 1/4 the diameter of L2. It carried the additional radio frequency (RF) to the animal's body and increased the radiating power of the transmitter.

In one modification of this circuit, C2 was detached from junction G and re-attached to junction D. It was utilized successfully for study of penned animals at Gorge

Creek.

To improve the range of transmission (better amplification at T2), and decrease the current drain of the transmitter by recycling some current, a PNP transistor (D30A5, General Electric) was selected for T1 (Fig. 2). One transmitter constructed utilizing the circuit, and a medium sized battery (RM 675, Mallory) gave clear records for 24 days. By reducing R2 to 100k ohms, the transmission range was increased from 2 to 5 m. Unfortunately, T1 requires relatively high base bias current to achieve satisfactory amplification. This resulted in high current drain and a shortened life for the transmitter. Therefore, this circuit was only used sparingly in cases where distance was not critical because chipmunks could not tolerate large batteries.

The final version of the transmitter is described in Fig. 3. It was best suited for the present study. The placement of the differential electrode (E) in a position which had no DC flow, but had a great potential difference, increased the amplification of T1 ten fold. This could also be accomplished by utilizing the circuit in Fig. 1 with the addition of a stabilizing resistor and a parallel capacitor to the emitter of T1. However, the physical size (1.8 x 1.0 cm in diameter) of the capacitor (64 m fd) necessary to accomplish such a differential, precluded its use in all but moderate sized or larger transmitters. The incorporation of

resistor R7 into the circuit (Fig. 3) prevented the malfunctioning of the transmitter due to overheating (thermal run away). The Darlington configuration of T2 and T3 isolated the amplification stage (T1) from the RF stage (T3). This resulted in good impedance matching between the two stages with little signal loss. It also eliminated the biasing problem encountered in the other circuits.

In all circuits, C3 and C4 allowed some negative feedback to stabilize the RF. The tank circuit, L2 and C5, acted also as an aerial. The value of C5 could be adjusted to modify the carrier frequency of the transmitter on the FM band. Fine tuning was accomplished by addition of ferrite chips during the potting stage.

All transmitters were first potted with 5 to 10 coats of Insl-X (Insl-X Products Corp., Yonkers, N.Y.). Then some were coated with epoxy resin. All were coated with a minimum of 3 layers of 1:1 paraffin and beeswax before implantation. All components except the battery fitted within the coil L2. The exposed portion of the EKG leads (E & F) extended 3 mm from opposing ends of the transmitter in the longest axis. The final characteristics of the transmitter including battery and potting were: size - 6 mm x 12 mm x 25 mm in length; weight - under 5 g; battery drain - less than .2 mA (in excess of 40 days theoretical life with a RM-675 battery); and a maximum recorded range of 25 m.

Transmitters were soaked in 1:1000 Roccol

(Alkylbenzyldimethylamonium chloride), prior to implantation within the abdominal cavity of the animals. Animals were anaesthetized with 50 mg/kg "im" Ketalar (Ketamine HCl) and 50 mg/kg "ip" Nembutal. The abdominal wall was shaved and wiped with Roccol. An incision 2 cm long was made in the skin and underlying wall of the abdominal cavity along the linea alba. The transmitter was inserted through the incision which was then closed by continual cross stitching with 4-0 opthalmic suture thread. Muscle layer and skin were sutured separately.

There were no discernable behavioral modifications observed 8 hours following the implantation. Within 24 hours after the operation, the incision had knitted sufficiently so as to make reopening extremely difficult. In 7 days, it was completely knitted over and indistinguishable from the surrounding shaved portion.

Pathological effects were evident upon autopsy if the transmitter was left implanted for a period greater than 2 months. Otherwise, the transmitter was free-floating in the abdominal cavity, and was covered with a thin sheet of connective tissue upon removal. With additional precautions for nonpyrogenic exposed surfaces, this time limit could be extended.

With the removal of the amplification stage and the attachment of temperature dependent oscilators at points A,B and D (Figures 1,2 and 3) such as those of Morhardt (1972)

or Wang (1972), the transmitter could also be converted into a temperature dependent transmitter.

OTHER CARDIOVASCULAR PARAMETERS

Remaining cardiovascular measurements were obtained from animals anaesthetized with 65 mg/kg "im" Ketalar followed by 65 mg/kg "ip" Nembutal.

It is worthy of note at this juncture, that five types of anaesthetic were initially utilized. Pentothal with Nembutal (Combutal) was found to be sufficient for short operations, but difficult to maintain during measurements longer than 1 hour. Chlorolose was abandoned shortly after the initial use, due to the difficulty in getting the compound into solution, and due to the repeated sickness of animals during recovery. Flourthane (halothane) was abandoned after it was discovered that the compound even in 1% air saturation, gave sufficient positive deflection to drive the G-2 analyzer off scale. Nembutal (Sodium pentobarbital) was used initially, and finally was combined with Ketalar (Ketamine HCl) as the best combination. This combination utilized approximately 60 mg/kg Ketalar "im" and approximately 65 mg/kg Nembutal "ip". The effect of the Ketalar was twofold. It was a rapid inducer (under 5 min), and it maintained a steady third stage anaesthesia without problems of synergistic effects upon second injection if prolonged anaesthesia was desired. However, it did not

prevent the decrease of body temperature which is associated with Nembutal anaesthesia. This trait is thought to have given rise to the low stroke volume (SV) calculations .

Cannulation techniques followed those of Popovic and Popovic (1960), Popovic et al. (1963), and Popovic and Berger (1968). Due to the small size of the vessels (carotid approximately 0.7 mm in diameter, jugular full, approximately 1.0 mm, collapsed, approximately 0.1 mm), polyethylene tubing (PE-10, Clay Adams Inc.) was initially used. This was soon abandoned, however, as the stiffness of the tubing and its small lumen rendered it prone to puncture the vessels and difficult to prevent clot formation within the cannula. Subsequently, PE-50 tubing (10 cm x 0.15 cm O.D.) was heated over a low voltage (8V, AC) heater made of a loop of nickel chromium wire to just melting and pulled to an O.D. similar to that of PE-10. The tubing was then bent to conform to the contour of the appropriate vessel by passing a piece of 40 gauge wire through the lumen of the tube; bending the tube to the desired contour; immersing the tube and wire in boiling water and subsequently letting it cool; and then removing the wire.

Attempts were also made to antithrombinize the walls of the cannula by first soaking the tubing in tridiodocialamine (Trilaurylamine, Matheson, Coleman and Bell), followed by soaking 24 hours in heparine solution, but with only limited success. Attempts to leave viscous fluid (tridiodocialamine)

in the tubing as suggested by Waeldele and Stoclet (1973) failed due to the susceptibility of the animals to the fluid.

From cannula implanted in the carotid artery, 0.02 ml of whole blood was drawn into a 2 cmm pipet (Hellige - perma line #578756), and washed into 5 cc of Drabkin's solution. Hemoglobin determinations were made by the cyanmethoglobin method (Drabkin, 1941, 1948; Drabkin and Austin, 1932, 1935-36a, 1935-36b; Crosby et al., 1954), optical density was measured at 540 nm by a Unicam Ultraviolet Spectrophotometer (model SP 1800, Canadian Laboratory Supplies). Next, 0.005 ml of whole blood was drawn into a red corpuscle dilution pipet, diluted 1:200. RBC counts were made utilizing the Levy and Levy-Hausser Corpuscle Counting Chamber (C.A. Hausser and Son, Phil. Pa.). Values are accurate to ±20% (Berkson, 1944). The cannula was then flushed with 0.1 ml of saline and sealed.

Blood volume determinations were made by the dye dilution technique first introduced by Keith et al. (1915). The dye T-1824 (Gregersen et al., 1935) was chosen because at its peak absorbance wave length (624 nm), it is little influenced by hemolysis (Gregersen, 1938), and also for its specific plasma binding characteristic with little washout within the first 30 minutes. In addition, once the plasma and bound dye are separated from the cells, the solution will remain stable for several hours if kept cool. Since the

peak absorbance wave length of T-1824 varies in different species (Allen et al., 1958), a standard absorption peak curve was therefore constructed. The peak for chipmunks occurred at 620 nm.

The dye, 0.1 ml of 10 mg/ml T-1824 solution in saline, was injected into the internal jugular vein via the jugular cannula. Ten to fifteen minutes later, samples were taken from the carotid cannula. Microhematocrit tubes (0.06 ml, Fisher Scientific Co. #2-668-60) were filled to 3/4, sealed with a hematocrit tube burner, and centrifuged for 5 to 10 minutes at 15,000 RPM by a Hematocrit Electrifuge (Chicago Surgical and Electrical Co.). After the determination of hematocrit, 0.03 ml of plasma was removed. To this was added 0.57 ml of saline. Its optical density was read at 620 nm on the Unicam.

In 20 animals (8 E.m.bo., and 12 E.a.lud.), RBC count and haemoglobin determinations were from blood obtained by cardiac puncture. The injection of dye and removal of blood for BV determinations were also via cardiac puncture.

Blood oxygen content and saturation curves were obtained by a modification of the polarographic technique described by Tucker (1967) and Kirk (1973). A radiometer blood gas analyzer (model #27 pH meter and #47 gas monitor, #E5046/0 oxygen electrode and #UTS13 circulating water bath, Radiometer, Copenhagen) was used to measure PO2 in ferricyanide solution. Blood samples of 0.01 ml each were

taken by heparinized micropipets (Fisher Scientific Co.) for A-V O2 difference measurement. Calculations converting PO2 to oxygen content followed Tucker (1967).

After A-V O2 difference measurements were completed, 0.7 ml of whole blood was removed from the carotid and placed in a tonometer for determination of haemoglobin oxygen saturation curves. A rotor (Fultork Labmotor, Fisher Scientific Co.) was connected via a piece of tygon tubing and a flexible copper coupling to the tonometer. The temperature of the bath surrounding the enclosed tonometer was kept constant at 37 ±0.1C by an open circulating water bath (Brinkman Instruments #KPR-D-1175, Canadian Laboratory Supplies). The exhaust tube from the tonometer chamber was connected to the inlet of a pH/blood gas analyzer (Instruementation Labatory Inc. #113) for PO2 measurement. Different PO2 values for blood equilibration in the tonometer were obtained by mixing compressed gasses of nitrogen and air. Blood PO2, PCO2 and pH were measured by the pH/blood gas analyzer.

Heart weights were obtained by sacrificing the animal with an overdose of Nembutal (7300 mg/kg). After opening the chest cavity, the pericardium was removed and as near as possible, all major blood vessels leading into and out of the heart were cut. The heart was then removed, cut into halves, briefly blotted dry to remove excess blood, and weighed to the nearest 0.5 mg.

STATISTICS

Analysis of Variance and Duncan's Multiple Range Test (Steel and Torrie, 1960) were used for comparison of estimated basal metabolic rate (EBMR), heart weight, haematocrit, haemoglobin concentration, and minimum HR in the TNZ of different subspecies or species of chipmunks. Analysis of covariance, Bartlett's chi square and Spearman's Correlation Coefficient (Sokal and Rohlf, 1969) were used for comparisons of thermal conductances and HR versus Ta. All other values were compared using the t-test or analysis of variance (Steel and Torrie, 1960; Weinberg and Schumaker, 1969). Differences were considered to be statistically significant whenever alpha or p value was less than 0.05. In all cases where reported or predicted values were included for comparison, ±20% of the value was used as the standard deviation at the 95% confidence level (Berkson, 1944; Weinberg and Schumaker, 1969).

RESULTS

CIRCADIAN RHYTHM

Two sets of typical hourly minimum oxygen consumption values in continuous 24 hour measurements are shown in Fig. 4A. There were 2 apparent peaks occurring in the normal "light" period of the holding quarters, even though the measurements were obtained while the animals were retained in a totally darkened animal chamber.

Linear regression calculation of the slopes of minimum oxygen consumption measured between 0 and 25C for "light" versus "dark" for each species had p values between less than .2 and less than 0.05 (Table 1A, Fig. 5-9). Measurements of all species combined indicated that minimum oxygen consumption below the TNZ during the "light" period was significantly higher than that during the "dark" (Table 1A).

In all four subspecies, the total oxygen consumed on a per hour basis during the "light" period was significantly higher than that consumed during the "dark" (Table 1B).

There were no significant interspecific differences between "light" and "dark" EBMR when compared on a per g or a per $kg^{0.75}$ basis (Fig. 10). However, all four subspecies had EBMR's which were significantly higher than their weight predicted values (Tables 2 and 3).

Typical hourly Tb recordings (Fig. 4B) from one individual continuously monitored at Ta's of 3, 10, and 22C illustrated the variability of Tb and mild hypothermia during the "dark" at Ta=3C. In all subspecies, the lowest Tb during the "light" period averaged 0.5 to 1.5C higher than that during the "dark" period (Fig. 11). All "dark" values at Ta=3C were significantly lower than those measured during the "light". In all cases except E.m.bo. at 25C, Tb1 in the "light" period was not significantly higher than the "light" values at Ta=3C. This suggests that the animals allowed a circadian fluctuation of their Tb's (Fig. 11). The maximum daily fluctuation recorded was 4.5C at a Ta of 3C (Fig. 4A).

Heart rate of E.a.lut. monitored at half hour intervals at Ta=17C and expressed as percent of minimum value, indicated that "light" values were significantly higher than those during the "dark" (Fig. 12A). Also, the mean heart rate in the "light" period was significantly higher than that during the "dark" (Fig. 12B).

OXYGEN CONSUMPTION

Minimum oxygen consumption values from both "light" and "dark" measurements are presented in Fig. 5-8 and 10. These values were computed both on a per g body weight, and on a per $kg^{0.75}$ basis. This later measure was taken to compensate for metabolic size (McNab, 1966; Kleiber, 1972). Minimum thermal conductance (TC) was calculated from minimum oxygen

consumption values below the estimated lower critical temperature (ELCT) during the "light" period. The values for the 4 subspecies were: 0.1138 (*E.m.bo.*); 0.1114 (*E.m.or.*); 0.1126 (*E.m.op.*); and 0.1126 ccO2/g-h-C (*E.a.lut.*) (Fig. 9A). There was no significant difference amongst these values ($F=9.3 \times 10^{-3}$, and 213 df). After correction for metabolic size, the corresponding values were: 54.4, 54.0, 50.4, and 52.1 ccO2/$kg^{0.75}$-h-C, also not significantly different from each other ($F=2.1 \times 10^{-1}$, 3 and 213 df) (Fig. 9B).

The ELCT's from "light" oxygen consumption measurements were 25.4 (*E.m.bo.*); 26.1 (*E.m.or.*); 24.3 (*E.m.op.*); and 23.5C (*E.a.lut.*) respectively, when computed from ccO2/g-h; and 25.3, 25.6, 24.7 and 23.7C respectively (Fig. 9) when corrected for metabolic size. ELCT was determined by the intersection of TC and EMBR.

Readings obtained between Ta's of 25.5 and 32C were used for computation of estimated basal metabolic rate (EBMR). There were no significant differences between "light" and "dark" EBMR's within each subspecies (Fig. 10). All subspecies had EBMR's significantly higher than their weight specific values (Tables 2 and 3). From analysis of variance and Duncan's multiple range test, the EBMR's of the alpine species (*E.m.or.* and *E.m.op.*) were significantly lower than those of the boreal species (*E.m.bo.* and *E.a.lut.*) both on a per g and a per $kg^{0.75}$ basis.

Minimum oxygen consumption measurements from animals maintained in the cold room were lower than those of non-acclimated animals between Ta's of 5 and 25C. However, the difference was not significant for any subspecies.

HEART RATE

The equations for minimum heart rate in the "light" period versus Ta between Ta's of 0 and 25C were 609 - 16.125Ta (*E.m.bo.*); 653 - 14.273Ta (*E.m.or.*); and 667 - 16.125Ta (*E.a.lut.*) (Fig. 13A). Mean minimum heart rates measured in the TNZ were 272 (*E.m.bo.*), 291 (*E.m.or.*), 267 (*E.m.op.*), and 336 (*E.a.lut.*) beats per min respectively for the four subspecies (Table 3). The values predicted from the equations in Fig 13A for the 3 subspecies were 273, 296, and 264 respectively at Ta=25C (Tables 4 and 5). The oxygen pulse (OP) which is defined as MR/HR, indicates the amount of oxygen delivered by each heart beat of the animal. The calculated OP's at 25C were 5.49×10^{-3} (*E.m.bo.*), 4.50×10^{-3} (*E.m.or.*), 4.48×10^{-3} (*E.m.op.*), and 5.56×10^{-3} (*E.alut.*) (Table 5 and Fig. 13B). The OP's at all Ta's were significantly lower for *E.m.or.* (alpine subspecies) than for *E.m.bo.* and *E.a.lut.* (boreal subspecies). There was no significant difference between *E.m.bo.* and *E.a.lut.* From the equation of Bartholomew and Tucker (1963), the % contribution of the increased heart rate to oxygen transport under increased metabolic demand is equal to:

$$\left[\frac{HR1 - HR2}{HR}\right] \div \left[\left\{\frac{HR1 - HR2}{HR2}\right\} + \left\{\frac{OP1 - OP2}{OP2}\right\}\right]$$

where:

HR1 is the heart rate in the changed state

HR2 is the initial heart rate

OP1 is the oxygen pulse in the changed state

and OP2 is the initial oxygen pulse.

The contribution of HR increased from 79% to 91% as Ta decreased from 20C to 5C.

OTHER CARDIOVASCULAR PARAMETERS

Results from anesthesized animals under thermal neutral Ta's are summarized in Tables 4 and 6. Arterial blood had a total saturation of 16-23% by volume and a P50 between 25-42mm Torr for all subspecies (Fig. 14). The mean A-V O2 differences were 5.6% (E.m.or.), 7.8% (E.m.op.), and 10.4% by volume (E.a.lut.), and mean cardiac outputs (CO) were 23.6, 16.5, and 16.2 cc blood/min and the calculated mean SV's were 0.057, 0.032 and 0.04cc/beat respectively (Table 6).

DISCUSSION

CIRCADIAN RHYTHMS AND SOME BIOLOGICAL IMPLICATIONS

From trapping records, it has been generally recognized that chipmunks are diurnal. However, attempts to demonstrate circadian rhythm in physiological parameters have not been met with success. For instance, Wunder (1972a) could not demonstrate any difference between "day" and "night" measurements of Tb, HR and O2 consumption. Pohl (1972) could only demonstrate a diurnal activity rhythm in 7 out of 15 E. sibiricus. He attributed this lack of consistency as due to the great variability amongst individual animals.

Experience gained from the present study indicates that chipmunks are extremely agile animals, and very sensitive to laboratory disturbance. In the presence of the investigator, the typical response of the animals was repetitive back-flips at a rate of approximately 1 to 2 per sec, hitting the floor and the top of the cage. In those individuals with EKG transmitter implants, the HR was observed to increase 2 to 3 times at the very instance when the investigator was approaching the cage. (See fig. 15 for examples of rapid EKG alterations.) Also, Tb (Tb1 monitored continuously with thermocouple implants) was observed to increase from 0.5 to 2C within 60 sec, and as much as 3C in 5 min when the animal was disturbed by gentle manipulation of its thermocouple leads, usually lasting from 10 sec to 2 min. Therefore, it

is not surprising that quantitative consistency was difficult to demonstrate in those previous studies because of the great sensitivity of chipmunks to captive conditions. The expression of some of the sensitivity and variability in a 24 hour period can be seen in Tb (Fig. 4B), oxygen consumption (Fig. 4A), and HR (Fig. 12) recordings.

In the present study, total oxygen consumed during the "light" period expressed as per hour was significantly higher than that during the "dark" suggesting a circadian rhythm of this parameter. Also, when heart rate was expressed as percent of minimum values during a 24 hour run, the "light" measurements were significantly higher than those during the "dark". Similar to that reported by Pohl (1972) for activity, the individual variability of these measured parameters was also great. However, due to large sample sizes used in the present study, and possibly the utilization of biotelemetry, it was possible to detect significant differences between "light" and "dark" measurements of these parameters.

When comparisons were made on minimum oxygen consumption from 0 to 25C between "light" and "dark" periods, the difference was only approaching significance (Table 1A and Fig. 5-8). The lack of a clear cut difference between "light" and "dark" values could be attributed to the difficulty in obtaining minimum values because of the great susceptibility of these animals to disturbances as

previously described.

The exhibition of a diurnal rhythm in activity and other physiologcal parameters is considered to be advantageous to the survival of chipmunks in nature. A labile Tb can reduce the daily energy budget required for precision thermoregulation, yet as the observed maximum Tb differential was less than 5C in any daily measurements and rewarming could take place in less than 10 minutes, the animal would not be incapacitated if movement was required. Work, such as that of Brown (1973), Heller (1971), Sheppard (1968) and Merideth (1974) suggests that Eutamias is able to reduce the metabolic cost of aggressive encounters by two methods: (1) diurnal activities to avoid encounters with nocturnal species utilizing similar food sources (Brown, 1973); and (2) definitive behavioral patterns within the genus Eutamias so that interspecific aggressive encounters are decreased.

The animals in the field pen also exhibited a circadian rhythm (Fig. 16). The heart rate, when reduced to percent of the value predicted at the appropriate Ta illustrated the circadian rhythm. This animal also exhibited a behavioral pattern which may be considered adaptive to its energy budget. This animal was seen on several occasions basking on one of the support beams. The temperature differential between the beam and the front of the pen was 5 to 10C higher on the beam during the sunbasking episodes. From the

relationship of minimum oxygen consumption and Ta (Fig. 8), the saving from this temperature differential could have amounted to 7 to 18%. This pattern was similar to that found in the laboratory study of Neal and Lustick (1974) on Tamias striatus. They were able to demonstrate that artificial radiation from a heat lamp resulted in a metabolic saving of approximately 20% at the same Ta than without the radiation.

With the exhibition of a circadian rhythm in activity and some physiological parameters, and the utilization of behavioral thermoregulation, the total energy budget of chipmunks can be reduced. This could be a significant contribution to their success in out-competing existing species in the general habitat as well as invading new territories.

OXYGEN CONSUMPTION

Measurements of flushing time are important to this and any other oxygen consumption analysis. It serves to ensure that readings taken do reflect the real metabolic state of the animal, rather than a transitory change which might not reflect the true metabolic response of the animal to a given situation.

An example of the possibility of such biased sampling can be found in Randolph (1973) in his study on shrews. In this study, the Ta was allowed to decrease continuously from 26 to -8C, while the oxygen consumption values were recorded

once every 5 min. An appreciation of the difficulties in maintaining most shrews in laboratory quarters, and a knowledge of the greatly increased mortality rates of these animals when utilized for experimentation, have swayed this researcher to try to condense as much information into the least possible amount of time. With such an approach however, Randolph has failed to realize that by his own measurements of the flow rates and the volumes of the metabolism chamber involved, the flushing time for 99% equilibrium was calculated to be about 60 minutes by the formula described by Lasiewski et al. (1966). The formula is $t = 4.6 \times V/D$, where t is the time in min, V is the chamber volume in cc, and D is the flow rate in cc/min. Randolph's use of the 5 minute values obtained during the continual Ta changes could greatly affect the outcome of the experiment and jeopardize the accuracy of his conclusions.

Minimum oxygen consumption showed that the "dark" period was lower than that during the "light" period below the ELCT (Fig. 5-8, Table 1A). However, there was no significant difference between "light" and "dark" measurements within the TNZ (Fig. 10). The EBMR for all subspecies was comparable to the values reported by Heller and Gates (1971), Morrison and Ryser (1951) and Wunder (1972a). They were greater than or equal to the weight specific values predicted by the equations of Morrison et al. (1959), McNab (1966) and Kleiber (1961) (Tables 2 and 3).

Only the "light" measurements of oxygen consumption were used for calculation of minimum TC. This was done mainly because the Tb of the animal decreased significantly during the "dark" period below Ta of 25C (Fig. 4B and 11).

Minimum thermal conductance values observed in the chipmunks were similar to those reported by Heller and Gates (1972), and were 72 to 85% of those predicted by weight according to the equation of Herreid and Kessel (1967). The thickness of fur in E.a. and E.m. (Heller and Gates, 1971) was between 0.8 and 0.9 cm, which was comparable to similar sized mammals living in arctic environments (Scholander et al., 1950). Under cold exposure, chipmunks curled into a very tight ball. In this posture, the surface to volume ratio approached minimum . The long guard hairs of the pelt were observed to be fully erect, thereby retaining a thick boundary layer of stagnant air. Under these conditions, heat loss was reduced to minimum. It is therefore reasonable to suspect that the observed depression of TC could be accounted for by the increased fur length and postural adjustments.

It is interesting to note that the alpine subspecies had significantly lower EBMR's than those of both subspecies from the boreal habitat (Fig. 2 and 3). Since the minimum TC's of all subspecies were also lower than their weight specific values, and since the TC of the alpine subspecies was equivalent to or lower than the boreal subspecies even

though their body weight was significantly lower; if the correction of TC was based on $cc O_2/kg^{0.505}$-h-C, then the MR's of the alpine subspecies would have been lower than those of the boreal subspecies at the same Ta. Such a metabolic saving could be significant in the daily energy budget of E.m.or. and E.m.op. inhabiting their relatively harsh alpine environments.

BODY TEMPERATURE

Wunder (1972a) did not observe any difference in Tb between "light" and "dark" periods at Ta=5C. The difference between Wunder's study and the present one could be due to the sampling techniques. Wunder (1972a) measured only the rectal temperature with a telethermometer probe, 45 to 60 sec after removal of the animal. In the present study, implanted thermocouples were used for Tb measurement. In view of the chipmunk's ability to increase their Tb 0.5 to 2C in 60 sec after disturbance, it is difficult to demonstrate a diurnal difference in Tb at Ta=5C without continuous measurement. The unexplainable drop in oxygen consumption reported by Wunder (1972a) at 5C could, therefore, be explained by the fact that Tb of the animals was below normothermic values during the "dark" period.

All subspecies of chipmunks maintained in the environment chambers were observed to exhibit hibernation between October 1972 and March 1973. Only 80% of the

individuals hibernated. On two separate occasions, one animal hibernated during the oxygen consumption measurement. The Ta was 15C. The calculated metabolic cost for 24 hours during hibernation was about 4.4% of its normothermic value at the same Ta. This suggests that the energy budget required by a normothermic animal to survive 24 hours could sustain a hibernating animal for 22 days.

CARDIOVASCULAR PARAMETERS

The data on heart rate suggests that there must be some significant alterations in the cardiovascular capacity of these animals. All subspecies studied have mean BHR's which were only 49 to 50% of their weight specific values as predicted by the equations of Stahl (1967) and Spector (1956) (Table 4 and Fig. 17). This finding is similar to that of Wang and Hudson (1971) for the eastern chipmunk, T. striatus. E.m.bo., E.m.or., and E.a.lut. value predicted by the linear regression equation for HR at 25C and E.m.op. from mean values, also had basal heart rates which were 87 to 94% of the values predicted by the equation of Hudson (Wang and Hudson, 1971) (Tables 4, Fig. 17).

The calculated OP was 5.49 (E.m.bo.), 4.50 (E.m.or.), 4.48 (E.m.op.), and 5.56 x 10^{-3} ccO2/beat (E.a.lut.) (Table 5). The computed weight specific OP by the combined equations of Stahl (1967), Spector (1956) and McNab (1966) were 2.79, 1.91, 1.88, and 2.41 x 10^{-3} respectively, which

are 50%, 42%, 42%, and 43% of the values observed in this study (Table 5). It is obvious from the relationship of MR to HR (see Morhardt, 1972 for some correlations in rodents) and from the equations: MR=HR x SV x (A-V O2 diff) and OP=SV x (A-V O2 diff) that the SV and/or the (A-V O2 diff) have increased sufficiently to compensate for the depression of HR in meeting the demand for oxygen by the animal.

Increased SV is dependent on two factors. The first of these is the size of the heart as indicated by its mass. The heart weights of the minimus subspecies were not significantly different from their weight specific values (Table 4). The heart weights of the luteiventris subspecies were significantly greater than predicted values. The second factor is blood volume. To support a doubling of SV, the venous return and available blood would also have to increased. Blood volumes of the minimus subspecies were not significantly different from weight specific values (Table 4). However, E.a.lut. had a lower blood volume than weight specific values (Table 4). It is therefore difficult to envisage the possibility that the increase of OP was due to increased SV. The blood volume (BV) overestimate due to dye extinction (Gregersen and co-workers, 1935, 1938, 1944) would further lessen this possibility.

From calculations of SV utilizing OP from Table 5 at 25C, and A-V O2 differences from 5.0 (human in supine position 4.0, standing 6.0, Iberall, 1972) to 10 (shrew has

a maximum A-V O2 difference of 13 vol %, Iberall, 1972; Bartels, 1966), the A-V O2 difference required for this SV would be approximately 7 volume % (Table 7). (If one assumes that this is a typical value for chipmunks, as indirect evidence seems to suggest little modification in SV in the chipmunks, one can calculate the theoretical values of A-V O2 difference from available data to see if they match the actual observations, using 0.07 cc/beat as SV and OP from Table 5 at Ta=25C.) In actual measurements, the A-V O2 difference ranged from 4.4 to 13.4 volume % even in anaesthetized animals. This seems to suggest that perhaps most of the modifications of OP come from factors contributing to increased A-V O2 difference.

A-V O2 difference is dependent on 2 major factors. The first is the specific characteristics of blood oxygen capacity. RBC and haematocrit values of chipmunks (Table 4) were not significantly different from values reported by Hall (1965) for seven species of rodents. Haemoglobin values (Table 4) tended to be greater than values reported by Hall (1965). Assuming the haemoglobin (Hb) is 100% saturated in the arterial blood, the theoretical oxygen capacity of blood can be obtained by multiplying Hb with 1.34 ccO2/g-Hb (Hall, 1965) plus O2 dissolved in plasma. The calculated values are 20.6 (E.m.bo.), 19.7 (E.m.or.), 18.9 (E.m.op.), and 22.4 ccO2/100 cc blood (E.a.lut.) respectively. Therefore, an A-V O2 difference of 7 would suggest an unloading of approximately 37% across the

capillary circulation. Actual measurement on Hb saturation indicated an average saturation of 80 to 90% at PO2 between 85 to 106 torr. It is therefore conceivable that the potential for increasing A-V O2 difference is relatively great in chipmunks. It is speculated that this could be due to enhanced Bohr effect (Schmidt-Neilsen and Larimer, 1958) and/or increased myoglobin affinity (Rand et al., 1973), both of which would be expected to be genetic.

The great potential for increasing A-V O2 difference in chipmunks is evidenced by the experiments where simultaneous measurements of oxygen consumption, HR, and arterial and venous blood oxygen content were made. Although all animals were anaesthetized, mean A-V O2 differences were 5.6 (E.m.or.), 7.8 (E.m.op.), and 10.4 volume % (E.a.lut.) respectively (Table 6). Since anaesthesia tended to reduce peripheral vasoconstriction, which would in turn decrease the venous return and increase HR, such combinations would probably result in lower SV values than in unanaesthetized animals. Even so, A-V O2 differences as high as 13.4 volume % were observed during anaesthesia. It is also worth noting that the measured mean values of A-V O2 difference were from anaesthetized chipmunks whose MR's were depressed to approximately 75% from normal, a situation where demand for oxygen is minimum. It is speculated that with greater oxygen demands, A-V O2 difference may increase considerably for better efficiency of oxygen utilization. The normally depressed BHR probably allows a wider range for the increase

of HR under adverse conditions. It is interesting to observe that the contribution of HR to increased MR below LCT (Table 5) increased steadily as the Ta became lower. This capability, together with the ability to expand A-V O2 difference, would appear to equip the animals with great cardiovascular reserves for aerobic metabolism.

Evidence to support this hypothesis comes from triplicate oxygen consumption measurements from an animal with an EKG transmitter running on the treadmill. The Ta was 12C and the speed was 7.5 m/min. During non-running episodes, the contribution of HR to MR was 85%. However, 2 min after the start of the running, the contribution of HR was reduced to 55 to 67%, yet MR increased 40%. The discrepancy would have to come from an increase of either SV and/or A-V O2 difference. This assumption also is supported by the observation that during repeated oxygen consumption and CO2 production recordings, the initial response to activity is an increase in RQ. The resultant CO2 washout would increase lung oxygen loading, and with increased CO2 production in the tissue, this would result in increased oxygen unloading in the tissues (Bartels, 1966).

Although it is highly speculative, the relatively large capacity of cardiovascular reserves in the chipmunks would certainly aid the animals in exhibiting their quick dash - still - quick dash activity bursts so familiar to people who frequent the forests.

CONCLUSIONS

Chipmunks have been found to be very agile animals living in diverse habitats which impose various types of stress on the inhabitants. They have evolved various behavioral and physiological strategies to overcome these pressures. Some of the strategies illustrated in this study included the following: conservation of daily energy budget by allowing circadian fluctuation of body temperature, oxygen consumption, heart rate, and concentrated activity periods during the day; the utilization of sunbasking to decrease the thermal gradient between body and ambient temperatures, thereby reducing energy expenditure; and the reduction of metabolic cost for thermoregulation below the LCT by the decrease of minimum thermal conductance. Chipmunks were also observed to have greater haemoglobin content than similar sized animals, and the oxygen capacity of blood was also greater. The stroke volume appeared to be normal as their heart sizes were comparable to other mammals of similar size. The A-V oxygen difference was greater than that of comparable sized animals, and this coupled with a normally depressed basal heart rate, provides the chipmunks with great cardiovascular reserves for aerobic metabolism.

It is suggested that, with present observations and other reports on adaptive behavioral patterns of chipmunks such as the reduction of interspecific aggression by

dominance hierarchy, the sum of these adaptations has enabled the chipmunks to radiate to their present distribution by successfully out-competing the existing species.

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TABLES

Table 1A. Slope of "light" (l) and "dark" (d) regression lines calculated by least squares from minimum oxygen consumption

Species	$cc O_2/g\text{-}h\text{-}C$		sig	$cc O_2/kg^{0.75}\text{-}h\text{-}C$		sig
	l	d	P<	l	d	P<
E. m. bo.	0.1138	0.0891	0.1	54.41	38.87	0.05
E. m. or.	0.1114	0.0882	0.2	53.99	39.46	0.13
E. m. op.	0.1126	0.0932	0.1	50.40	42.98	0.13
E. a. lut.	0.1126	0.0884	0.05	52.35	39.12	0.05
All spec.	0.1123	0.0884	0.05	52.35	40.08	0.05

Table 1B. Average total "light" (l) and "dark" (d) oxygen consumption per hour calculated from weighed chart recordings.

Species	$cc O_2/g\text{-}h$		sig	Ta	$cc O_2/kg^{0.75}\text{-}h$		sig
	l	d	P<	C	l	d	P<
E. m. bo.	3.64	2.50	0.01	16	1737	1190	0.01
E. m. or.	4.02	2.92	0.01	15	1782	1294	0.01
E. m. op.	4.31	2.41	0.01	19	1874	1073	0.01
E. a. lut.	3.30	2.75	0.01	17	1530	1291	0.02

Table 2. Comparison of Minimal Metabolic Rates (EBMR) in the Thermal Neutral Zone (TNZ). F ratio is 4. *=(P<0.05).778 at 3 and 161 degrees of freedom.

Species	N	sig			$\bar{X}$ M. R.	S. E.	interspecific significance	
		1	2	3	O2/g-h			
E.m.bo.	47	*	*	*	1.78	±0.06	*	-
E.m.or.	36	*	*	*	1.64	±0.04	-	*
Predicted[1]					1.26	±20%=±0.25		
Predicted[2]					1.28	±20%=±0.26		
Predicted[3]					1.31	±20%=±0.26		
E.m.op.	38	*	*	*	1.50	±0.03	-	*
E.a.lut.	44	*	*	-	1.69	±0.06	*	-

Table 3. Comparison of Minimal Metabolic rates (EBMR) in the Thermal Neutral Zone (TNZ) to the 3/4 power of the body weight. F ratio =4.0295 at 3 and 161 degrees of freedom.

Species	N	sig			$\bar{X}$ M. R.	S. E.	interspecific significance	
		1	2	3	$O2/kg^{0.75}$-h			
E.m.bo.	47	*	*	*	839	±28	*	-
E.m.or.	36	*	*	*	752	±19	-	*
Predicted[1]					596	±20%=±119		
Predicted[2]					605	±20%=±121		
Predicted[3]					620	±20%=±124		
E.m.op.	38	*	*	*	698	±10	-	*
E.a.lut.	44	*	*	*	828	±29	*	-

1 Morrison, 1958: M. R.= 3.8 $W^{-0.27}$
2 McNab,1966: M. R.= 3.4 $W^{-0.25}$
3 Kleiber, 1961: M. R.= 70 $W^{-0.25}$

Table 4. Cardiovascular parameters

Parameters	E.m.bo.	E.m.or.	E.m.op.	E.a.lut.	E.m.lud.
mean Ht.wt.	0.3364	0.2929	0.2654	0.4802	0.4716
mean wt.	54.66	44.94	44.60	61.61	58.28
n	16	5	8	14	13
SE	0.0119	0.0183	0.0166	0.0298	0.0122
Predicted [1]	0.3360	0.2773	0.02753	0.3780	0.3576
Sig.	NS	NS	NS	5%	5%
Predicted [2]	0.3330	0.2748	0.2728	0.3745	0.3543
Sig.	NS	NS	NS	5%	5%
mean Hct.	47.5	43.9	45.5	50.3	42.0
n	10	6	4	11	8
SE	1.0	1.6	2.3	1.7	0.6
Predicted [3]	39-44	39-44	39-44	39-44	39-44
Hb.	15.4	14.7	14.1	16.7	13.7
(g/100cc)					
mean wt.	56.72	44.2	45.8	61.6	57.9
n	9	8	5	7	12
SE	0.4	0.9	0.8	0.2	0.8
Predicted [4]	13-15	13-15	13-15	13-15	13-15
Hb x 1.34	20.6	19.7	18.9	22.4	18.3
BV (cc)	4.2	3.8	4.1	3.8	4.0
mean wt.	57.4	46.8	55.3	61.9	58.1
n	6	5	4	4	4
SE	0.4	0.9	0.8	0.2	0.8
Predicted [5]	3.6	3.1	3.6	3.9	3.6
Sig.	NS	NS	NS	NS	NS
RBC	8.77	7.78	9.61	7.71	8.36
($x10^6$/cmm)					
n	11	6	4	4	12
SE$x10^6$/cmm	0.32	0.87	0.31	0.79	0.21
mean HR	272	284	273	336	
(TNZ)					
mean wt.	53.7	43.03	39.0	58.0	
n	10	28	18	7	
Predicted [6]	511	521	523	492	
%of Pred.	53	49	52	55	
at 25C					
HR-Ta(equ)	273	296	273	273	
Predicted [7]	308	314	315	296	
% of Pred.	89	94	87	92	

1. Stahl, (1967): $Ht.wt.(g) = 5.8\ W(kg)^{0.98}$
2. Spector, (1956 and Adolph, (1949): $Ht.wt. = 0.0066W(g)^{0.98}$
3. Hall, (1969): 38.9 - 43.7 %
4. Hall, (1969): 12.6 - 15.2 g/100cc blood
5. Stahl, (1967): $BV = 65.6W(kg)^{1.02}$
6. Stahl, (1967): $HR = 241W(kg)^{-0.25}$
7. Wang and Hudson, (1971): $HR = 816W(g)^{-0.25}$

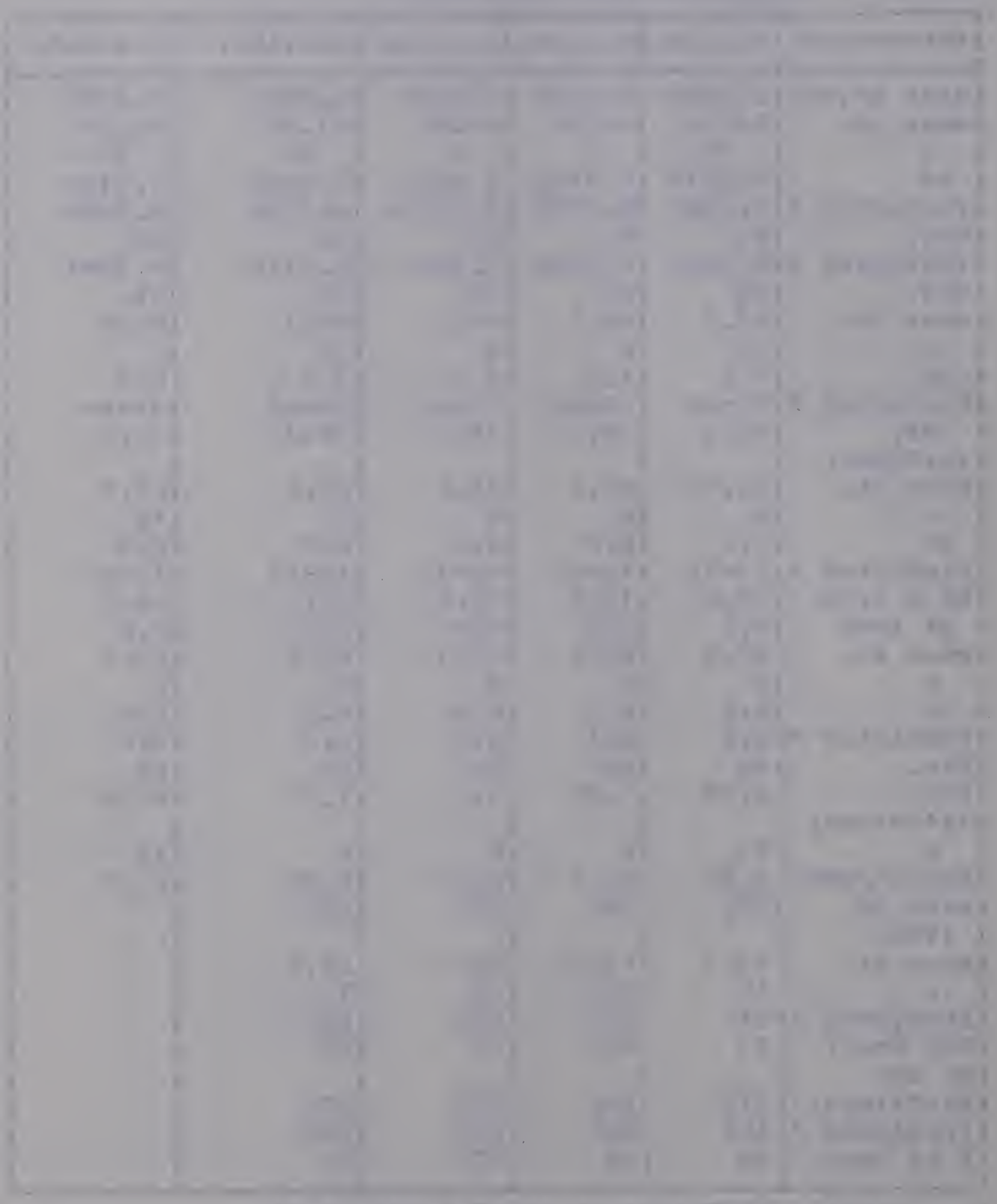


Table 5. OP and HR contribution to increased MR.

Species	Ta	O2/h	HR/min	OPx10³	Cont .
E. m. bo.	5	203	542	6.24	88
	10	175	475	6.12	87
	15	146	407	5.98	85
	20	118	340	5.78	82
	25	90	273	5.49	0
			Theoretical OP[1] at 25C=2.79		
E. a. lut.	5	218	586	6.19	91
	10	185	506	6.09	90
	15	153	425	5.97	89
	20	121	345	5.84	85
	25	88	264	5.56	0
			Theoretical OP[1] at 25C=2.41		
E. m. or.	5	182	581	5.21	86
	10	157	510	5.13	84
	15	131	439	4.97	82
	20	106	367	4.80	79
	25	80	296	4.50	0
			Theoretical OP[1] at 25C=2.41		
E. m. op.	TNZ	68	273	4.48	0
			Theoretical OP[1] at 25C=1.88		

1. Stahl, (1967); Spector, (1956) and McNab, (1966).

Table 6. Comparison of HR; A - V O2 difference, CO and SV.

Species	mean A-V O2 vol %	SE	mean HR b/min	SE	mean CO cc/min	SE	mean SVx10² cc/b	SE
E.m.or.	5.6	0.7	415	25.5	23.56	4.6	5.7	8.3
E.m.op.	7.8	1.0	514	14.5	16.5	8.2	3.2	2.0
E.a.lut.	10.4	1.7	406	5.5	16.2	1.3	4.0	3.5

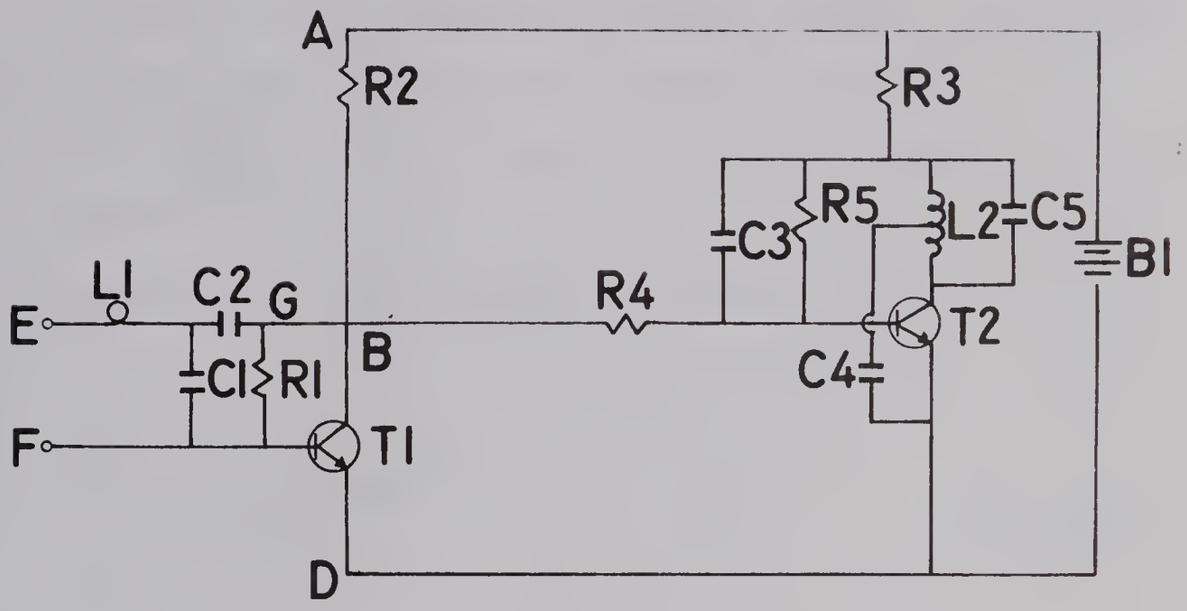
Table 7. SV calculations utilizing OP at 25C.

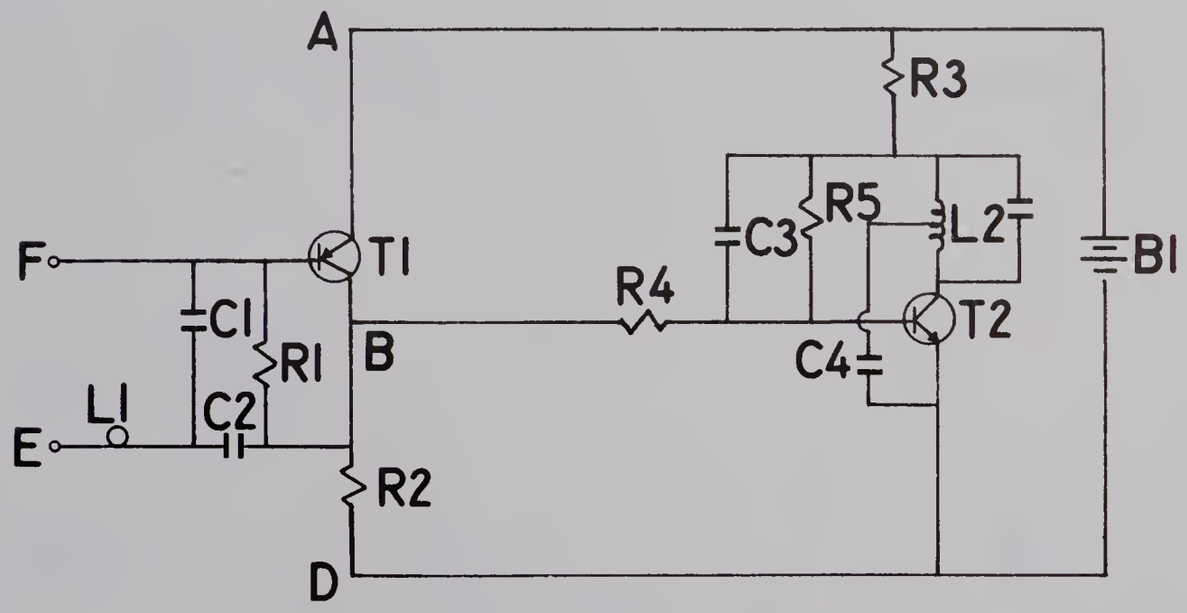
Species	OPx10²	A-V O2	SV
E.m.bo.	5.49	5	0.110
		6	0.092
		7	0.078
		8	0.069
		9	0.061
		10	0.055
E.m.or.	4.50	5	0.090
		6	0.075
		7	0.064
		8	0.056
		9	0.050
		10	0.045
E.m.op.	4.48	5	0.090
		6	0.075
		7	0.064
		8	0.056
		9	0.050
		10	0.045
E.a.lut.	5.56	5	0.111
		6	0.093
		7	0.079
		8	0.070
		9	0.062
		10	0.056

FIGURES

Figures 1;2 and 3. Wiring diagrams for EKG transmitters. All components are available from Newark Electronics, Chicago. All resistances are in ohms and all capacitances are in pfd.

Component code	Circuit 1 upper	Circuit 2 center	Circuit 3 lower
B1	RM-675	RM-675	RM-675
C1	12-18	18-33	12-22
C2	10,000	NA	500,000
C3	82-120	100	82-120
C4	82-120	100	82-120
C5	12-18	18-33	12-27
C6	NA	NA	10,000
L1	1T 2mm	NA	NA
L2	3T 10mm	3T 10mm	3T 10mm
R1	1M	1M	1M
R2	100-110K	1M	36-100K
R3	2.4-11K	11K	1.0K
R4	1.0-2.4K	2.4K	0
R5	7.5-11K	100K	1M
R6	NA	NA	0
R7	NA	NA	1.0-2.4K
T1	D26E-1/5	D30A-5	D26E-1
T2	D26G-1	D26G-1	D26E-1
T3	NA	NA	D26G-1


A
R2
R3
C3
R5
L2
C5
B1
L1
C2
G
R4
E
T2
B
C1
R1
C4
F
T1
D


A
R3
C3
R5
L2
B1
F
T1
R4
T2
C1
R1
B
C4
L1
C2
E
R2
D

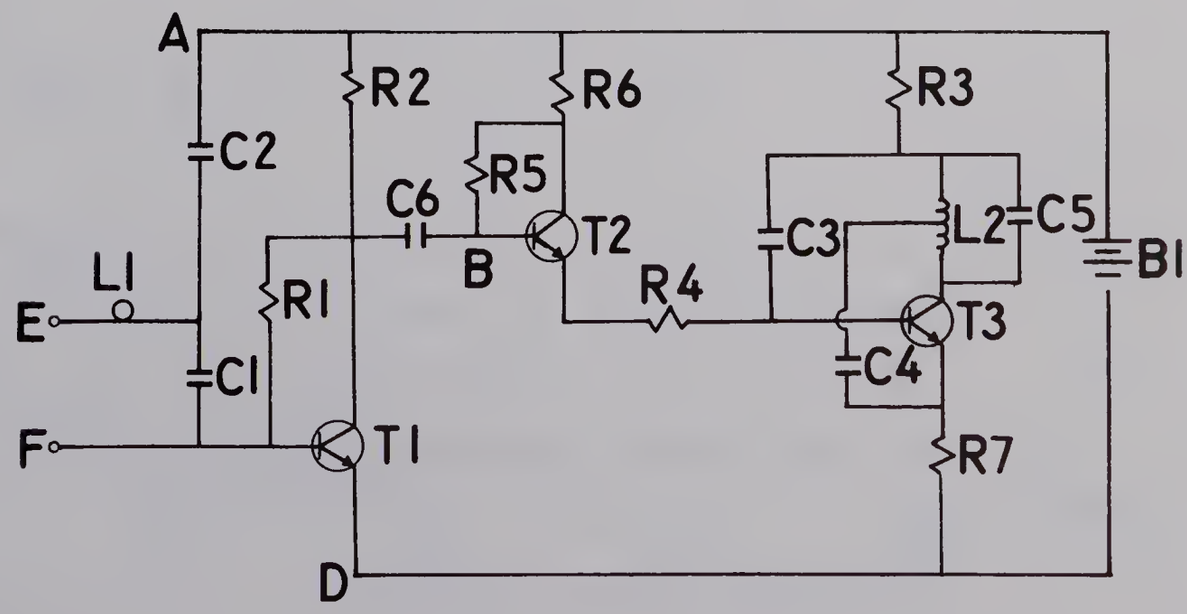

A
R2
R6
R3
C2
R5
C6
T2
C3
L2
C5
B1
L1
B
R4
R1
E
T3
C1
C4
F
T1
R7
D

Figure 4A. (upper) Oxygen consumption from continual records utilizing the minimal stabilized recording in each one hour period under continuous darkness at Ta=19C. Black bars indicate the "dark" period of the holding quarters. Arrow represents the start of the run.

Figure 4B. (lower) Body temperature (Tb1) from continual records utilizing the temperature on each hour at three Ta's under continuous darkness. Data from the same animal. Arrow represents the start of the run.

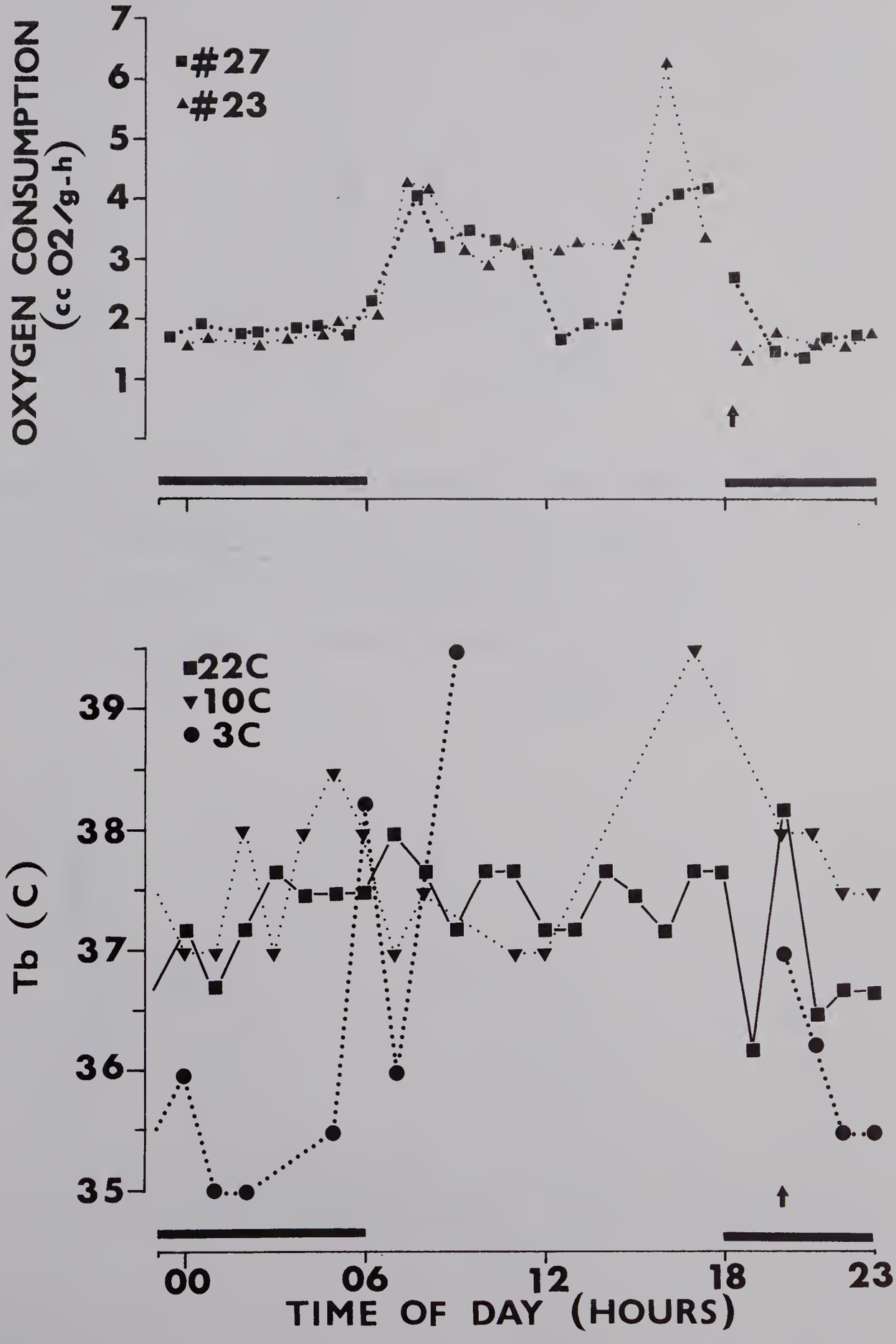

OXYGEN CONSUMPTION
(cc O2/g-h)
7
6
5
4
3
2
1
#27
#23
22C
10C
3C
39
38
37
36
35
Tb (C)
00
06
12
18
23
TIME OF DAY (HOURS)

Figure 5. Minimum oxygen consumption of E.m.bo. at different Ta's under "light" (○ ;120 measurements,n=12) and "dark" (▲ ;90 measurements,n=12) phases of the photoperiod, expressed as per g (upper) and per $kg^{0.75}$ (lower).

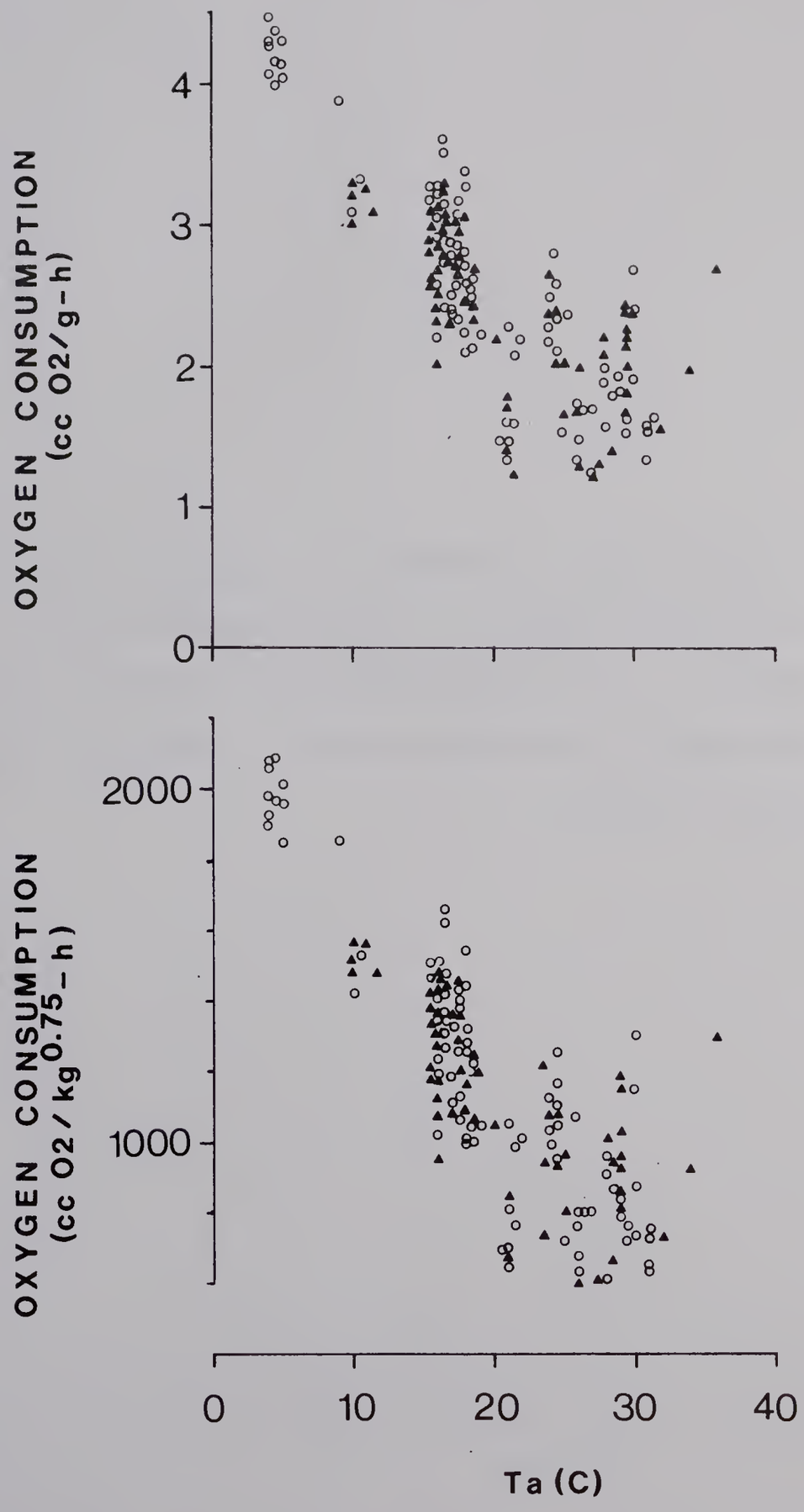

OXYGEN CONSUMPTION
(cc O2/g-h)
0
1
2
3
4
OXYGEN CONSUMPTION
(cc O2 / kg0.75_ h)
1000
2000
0
10
20
30
40
Ta (C)

Figure 6. Minimum oxygen consumption of E.m.or. at different Ta's under "light" (○ ;29 measurements,n=11) and "dark" (▲ ;43 measurements,n=11) phases of the photoperiod, expressed as per g (upper) and per $kg^{0.75}$ (lower).

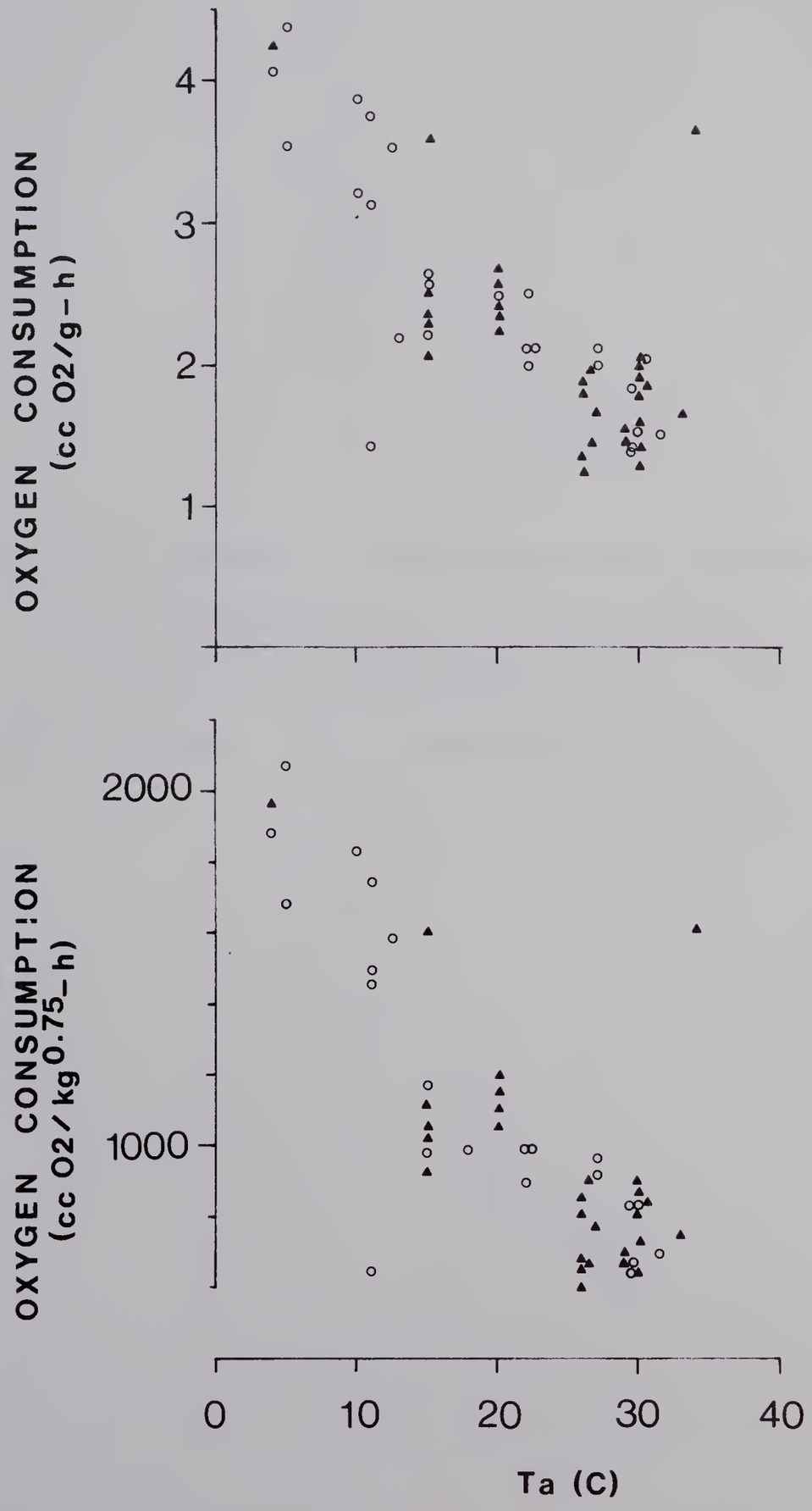
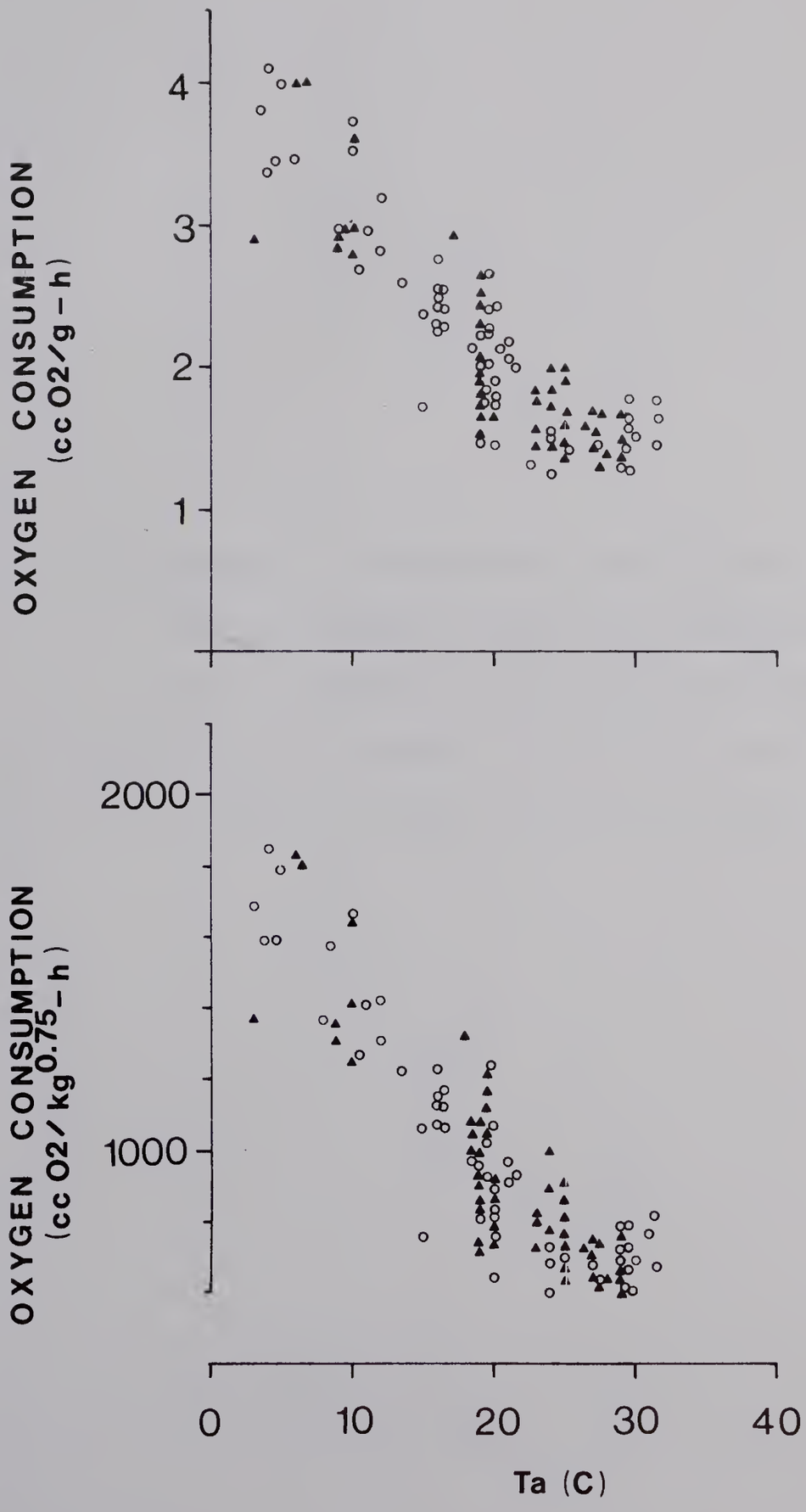

OXYGEN CONSUMPTION
(cc O2/g-h)
4
3
2
1
OXYGEN CONSUMPTION
(cc O2/kg0.75-h)
2000
1000
0
10
20
30
40
Ta (C)

Figure 7. Minimum oxygen consumption of E.m.op. at different Ta's under "light" (○ ;73 measurements,n=21) and "dark" (▲ ;85 measurements,n=21) phases of the photoperiod, expressed as per g (upper) and per $kg^{0.75}$ (lower).


OXYGEN CONSUMPTION
(cc O2/g-h)
4
3
2
1
OXYGEN CONSUMPTION
(cc O2/ kg^0.75 - h)
2000
1000
0
10
20
30
40
Ta (C)

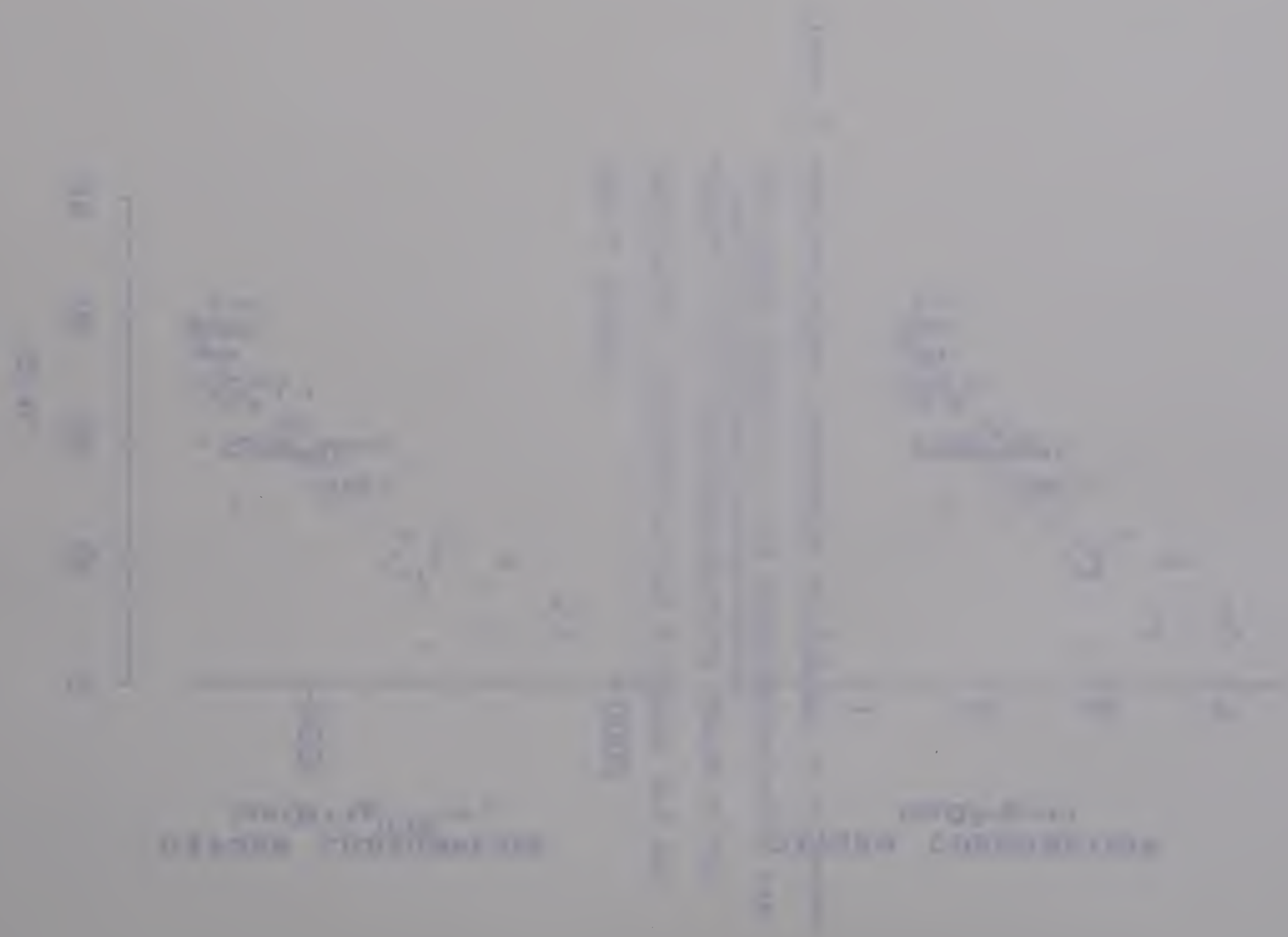
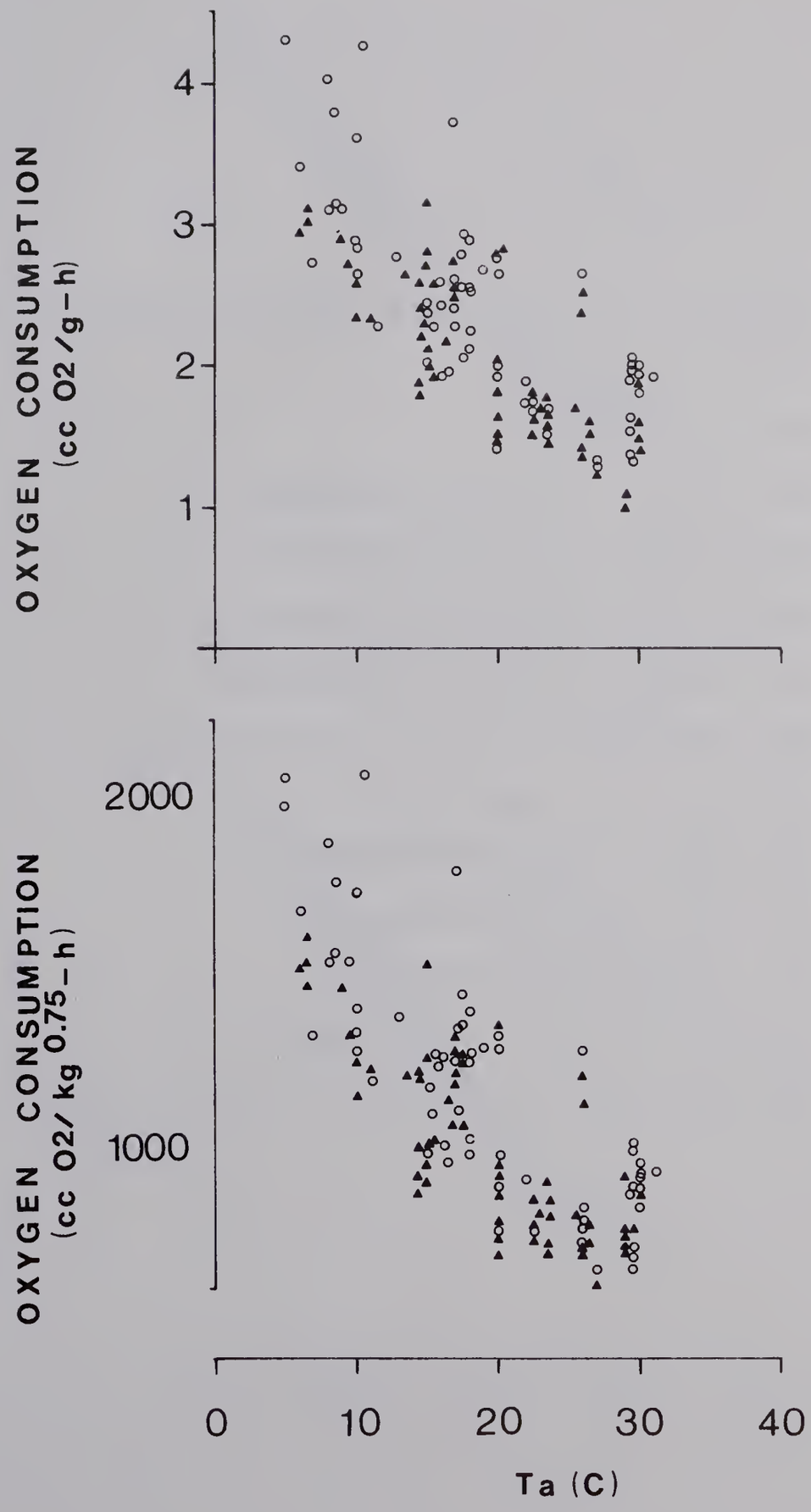


Figure 8. Minimum oxygen consumption of E.a.lut. at different Ta's under "light" (○ ;84 measurements,n=23) and "dark" (▲ ;88 measurements,n=23) phases of the photoperiod, expressed as per g (upper) and per $kg^{0.75}$ (lower).


OXYGEN CONSUMPTION
(cc O2/g-h)
4
3
2
1
OXYGEN CONSUMPTION
(cc O2/ kg 0.75-h)
2000
1000
0
10
20
30
40
Ta (C)

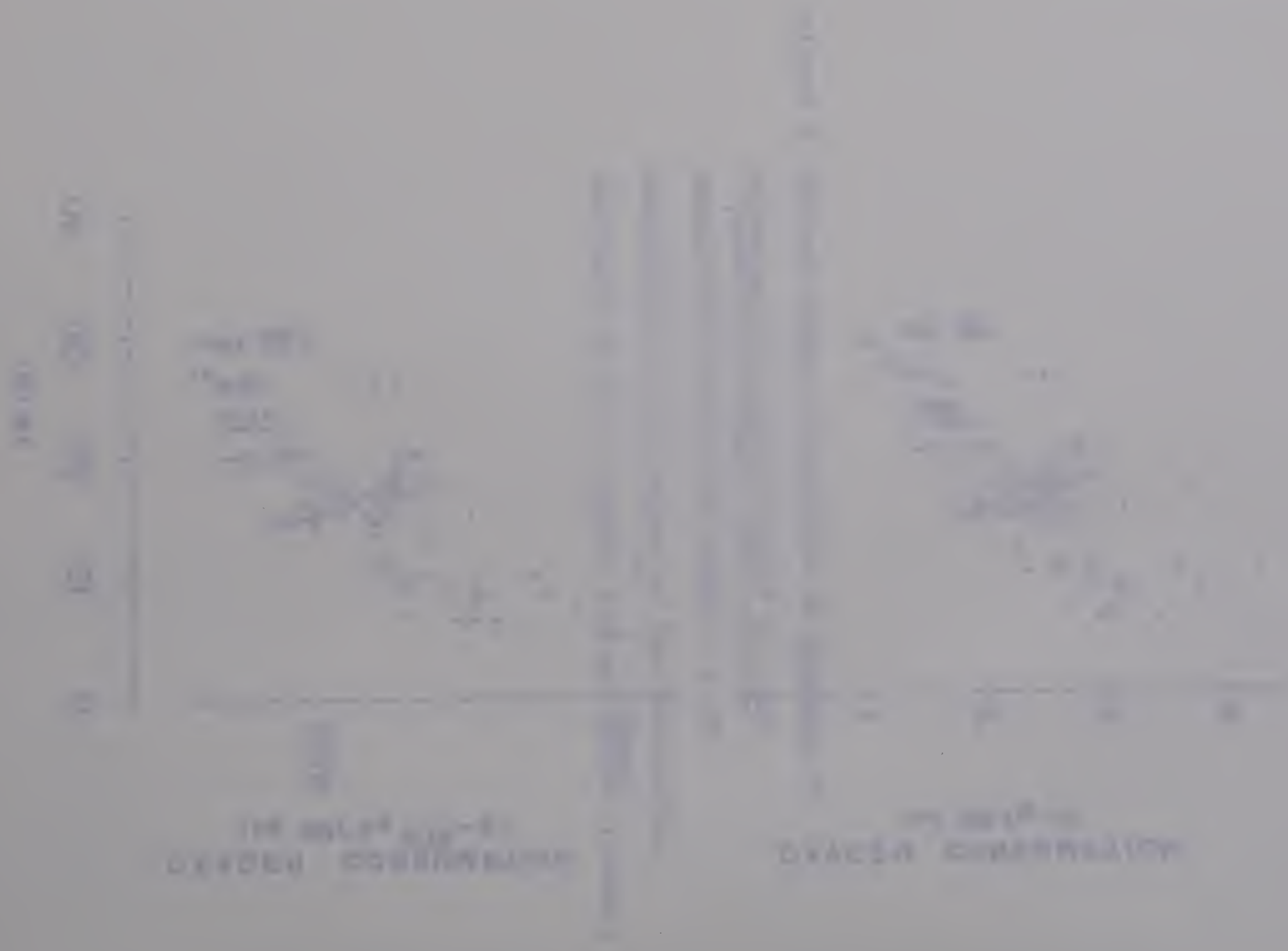


Figure 9. Minimum TC ("light" only) and BMR ("light" and "dark") comparisons

The equations are:

MR = 4.6772 - 0.1138 Ta (E.m.bo.)

MR = 4.5413 - 0.1114 Ta (E.m.or.)

MR = 4.2506 - 0.1126 Ta (E.m.op.)

MR = 4.3476 - 0.1126 Ta (E.a.lut.)

when expressed on a per g basis (upper),

and:

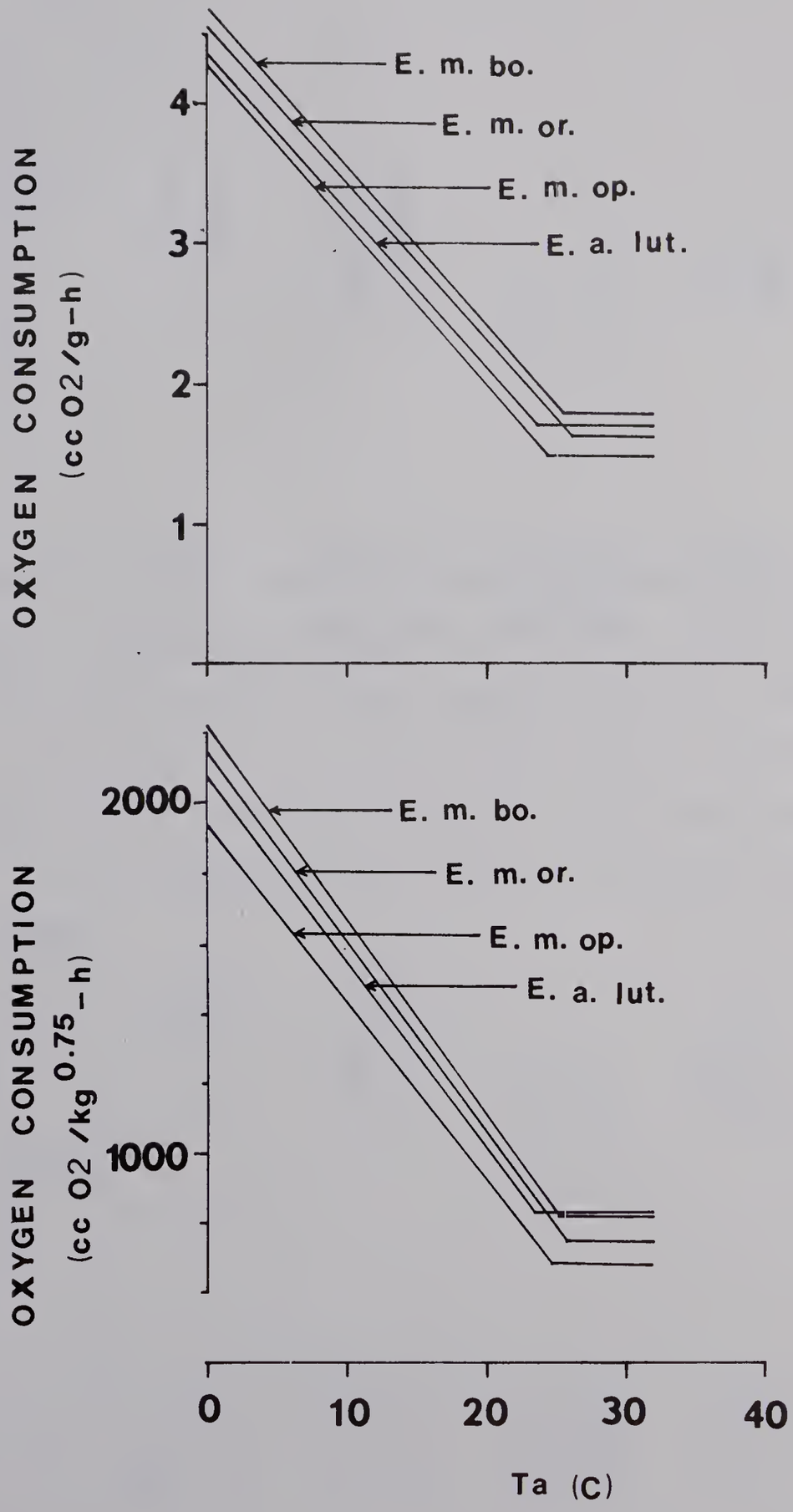
MR = 2213 - 54.41 Ta (E.m.bo.)

MR = 2132 - 53.99 Ta (E.m.or.)

MR = 1932 - 50.40 Ta (E.m.op.)

MR = 2065 - 52.09 Ta (E.a.lut.)

when expressed on a per $kg^{0.75}$ basis (lower).


OXYGEN CONSUMPTION
(cc O2/g-h)
4
3
2
1
E. m. bo.
E. m. or.
E. m. op.
E. a. lut.
OXYGEN CONSUMPTION
(cc O2/kg 0.75 -h)
2000
1000
E. m. bo.
E. m. or.
E. m. op.
E. a. lut.
0
10
20
30
40
Ta (C)

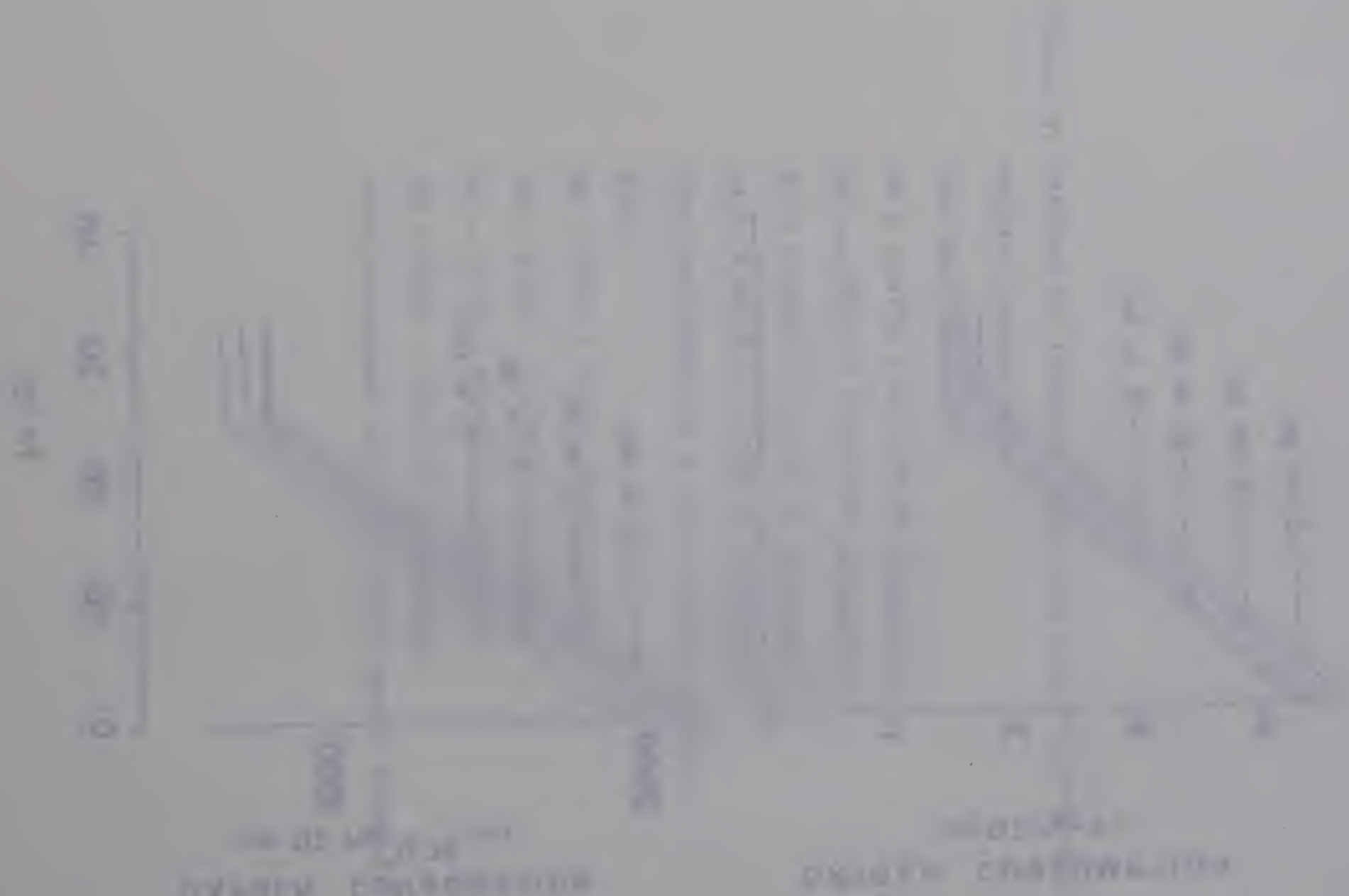
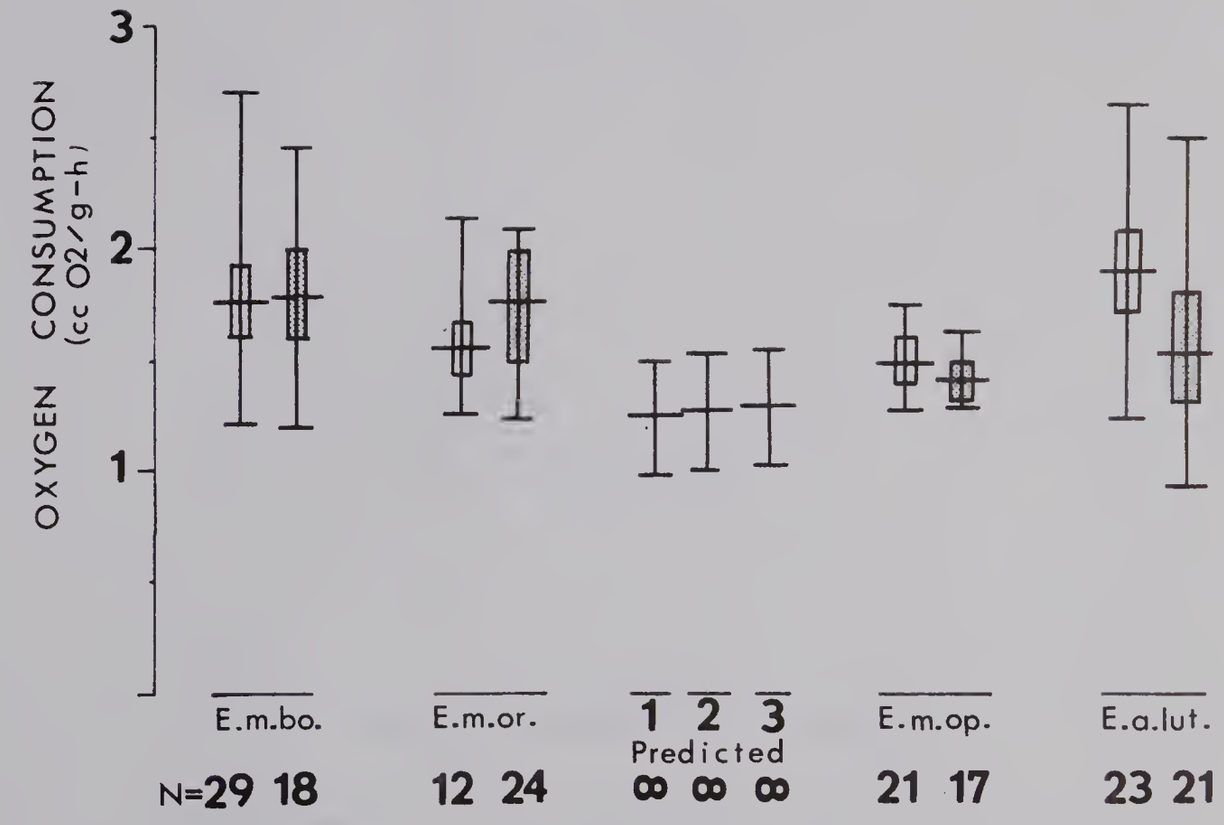
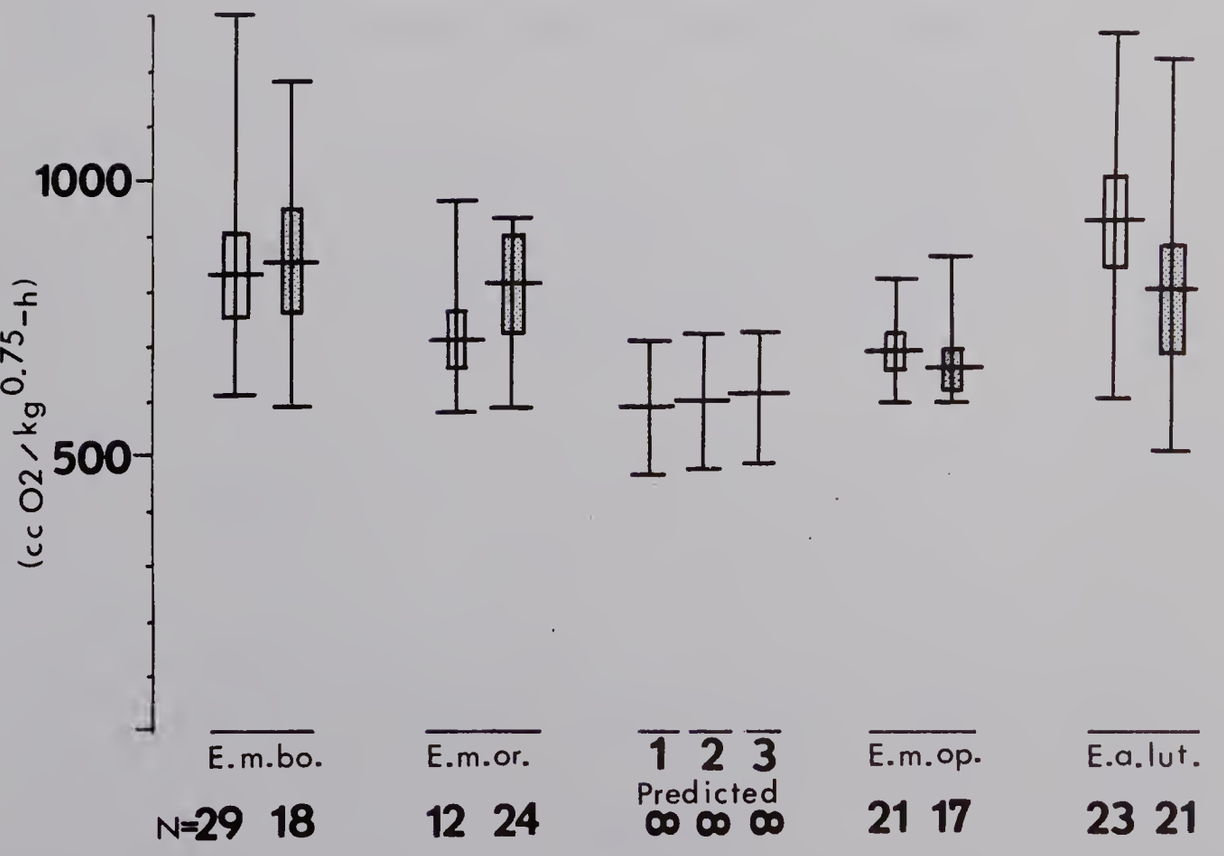


Figure 10. EBMR comparisons during "light" (open) and "dark" (shaded) photoperiods expressed on a per g (upper) and per $kg^{0.75}$ (lower) basis. End bars give the ranges. Center bars are the means. The boxes represent the 95% confidence limits of the mean. End bars on predicted values represent ±20%.


OXYGEN CONSUMPTION
(cc O2/g-h)
3
2
1
E.m.bo.
E.m.or.
1 2 3
Predicted
E.m.op.
E.a.lut.
N=29 18
12 24
8 8 8
21 17
23 21


(cc O2 / kg 0.75 -h)
1000
500
E.m.bo.
E.m.or.
1 2 3
Predicted
E.m.op.
E.a.lut.
N=29 18
12 24
8 8 8
21 17
23 21

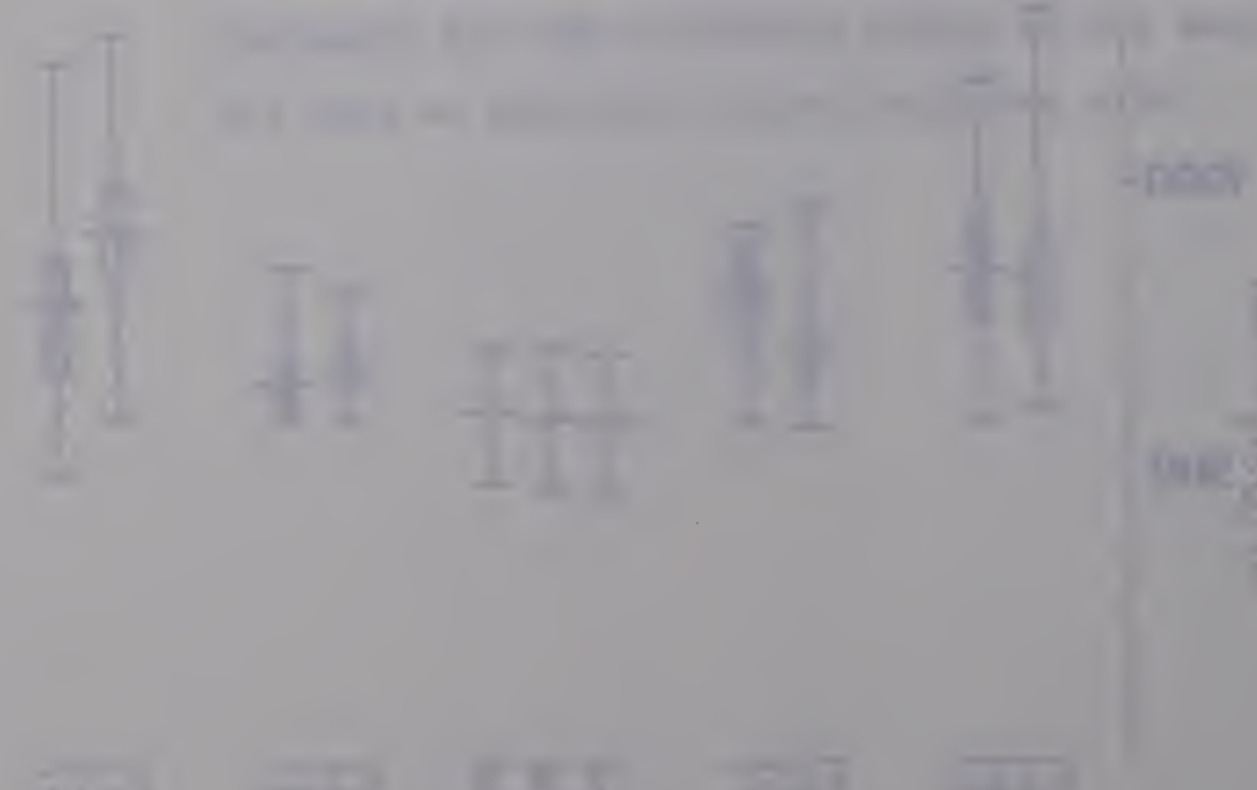


Figure 11. Comparisons of "light" (○) and "dark" (▲) period Tb's (Tb1), utilizing the 3 lowest Tb's per animal per run within each photoperiod (n=2 for each subspecies).

Predicted values are for a 50g animal by the equations of

1 Morrison (1958): $MR=3.8W^{-0.27}$;

2 McNab (1966): $MR=3.4W^{-0.25}$;

3 Kleiber (1961): $MR=70W^{-0.25}$.

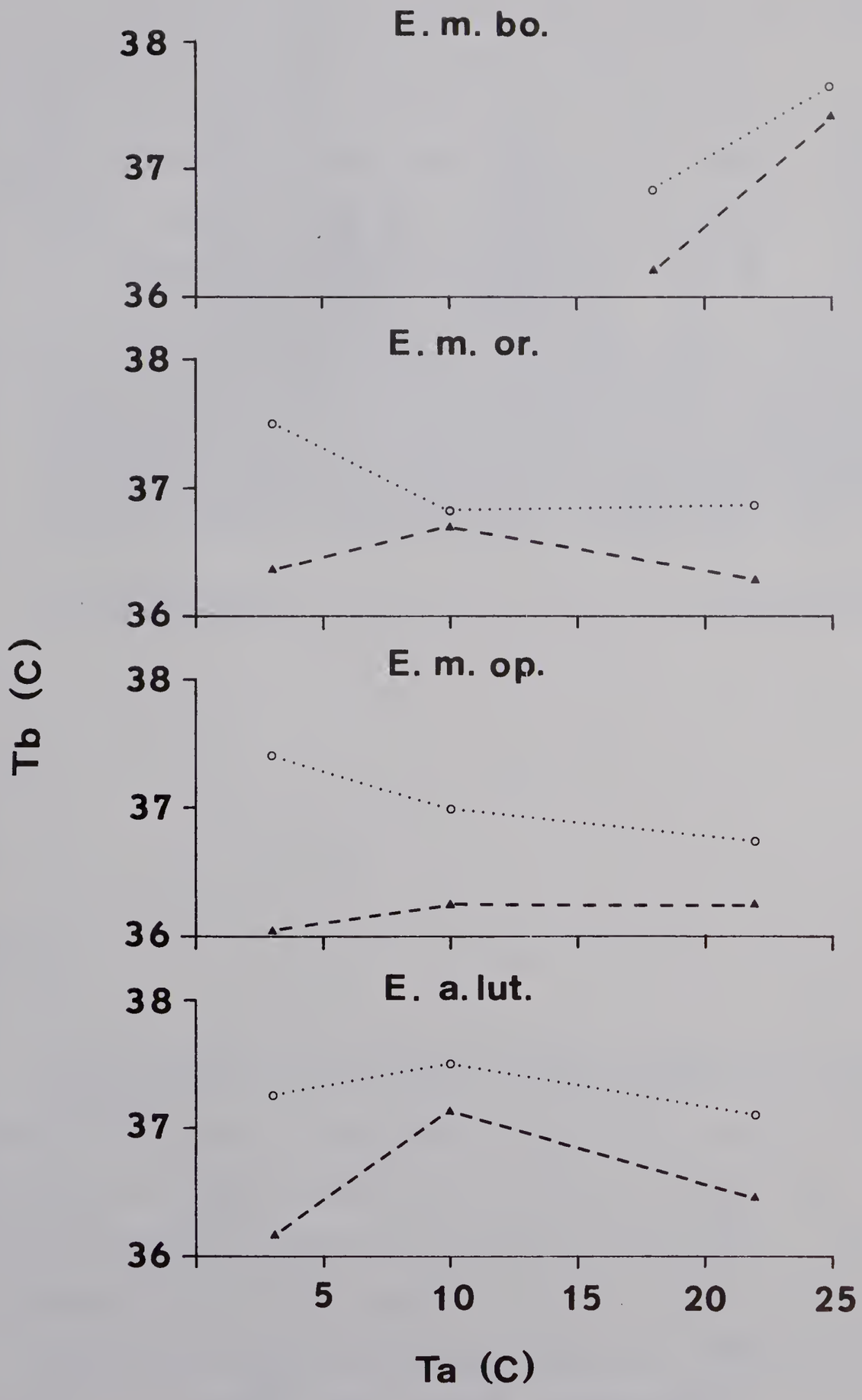

E. m. bo.
E. m. or.
E. m. op.
E. a. lut.
38
37
36
Tb (C)
5
10
15
20
25
Ta (C)

Figure 12A. (upper) Shade diagram illustrating the variability in heart rate. Each measurement is expressed as a percentage of the minimum heart rate of the individual animal per Ta in each run. Black bars indicate the "dark" period of the holding quarters. (169 measurements;n=8)

Figure 12B. (lower) Diurnal fluctuation of mean heart rate per interval expressed as percentage of the minimum heart rate (as above, 12A) to eliminate individual variation. Ta =17C. F ratio of "light" versus "dark" is 4.67 as 1 and 167 df. Horizontal line represents population mean.

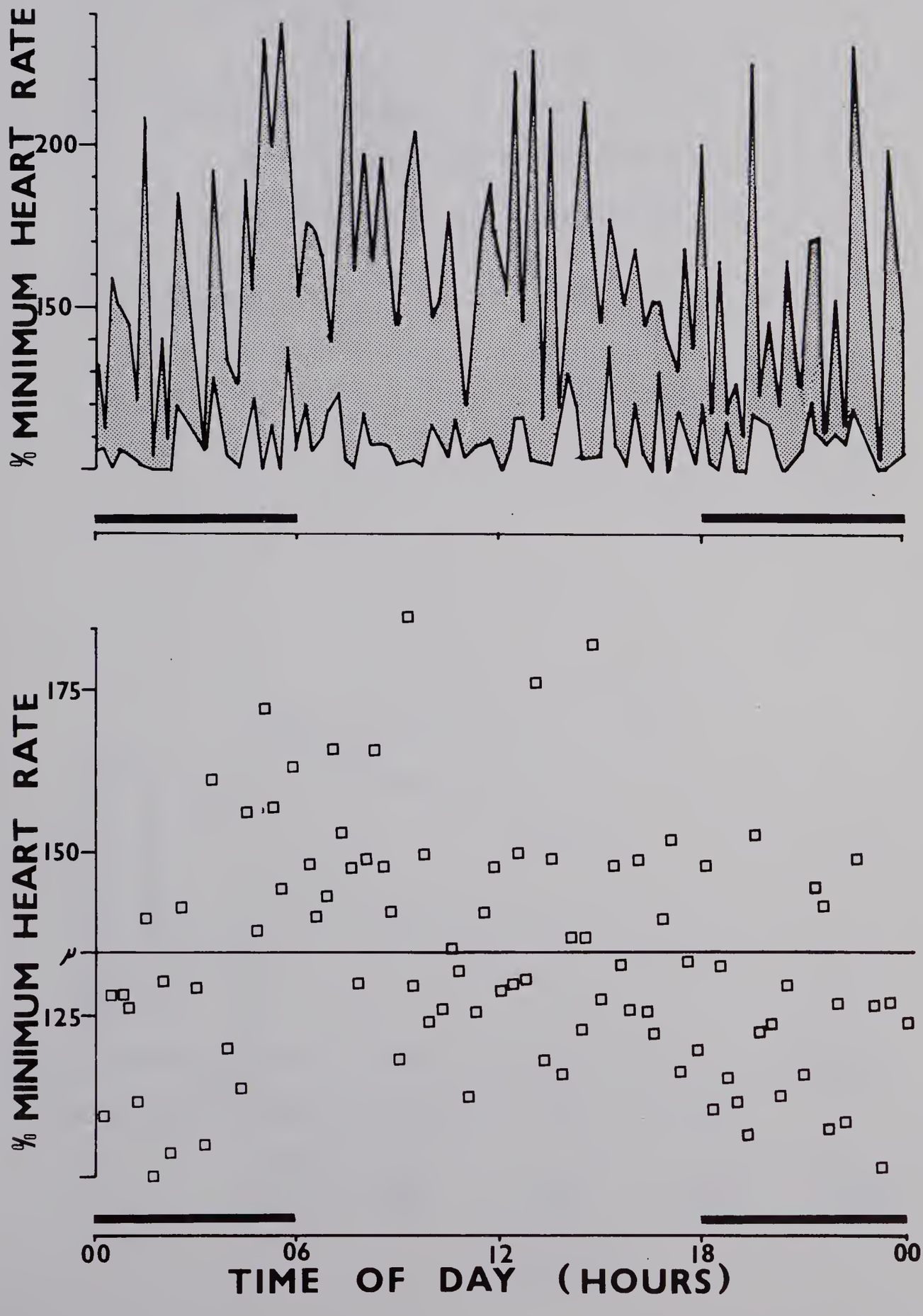

% MINIMUM HEART RATE
200
150
% MINIMUM HEART RATE
175
150
μ
125
00
06
12
18
00
TIME OF DAY (HOURS)

Figure 13A. (upper) Minimum HR versus Ta calculated from the 3 lowest HR's per run at each Ta.

The equations are:

HR = 609 - 13.443 Ta (*E.m.bo.*, N=18)

HR = 653 - 14.273 Ta (*E.m.or.*, N=61)

HR = 667 - 16.125 Ta (*E.a.lut.*, N=62)

Figure 13B. (lower) Calculated OP's from the equations of MR versus Ta (Fig. 9A) and HR versus Ta (Fig. 13A).

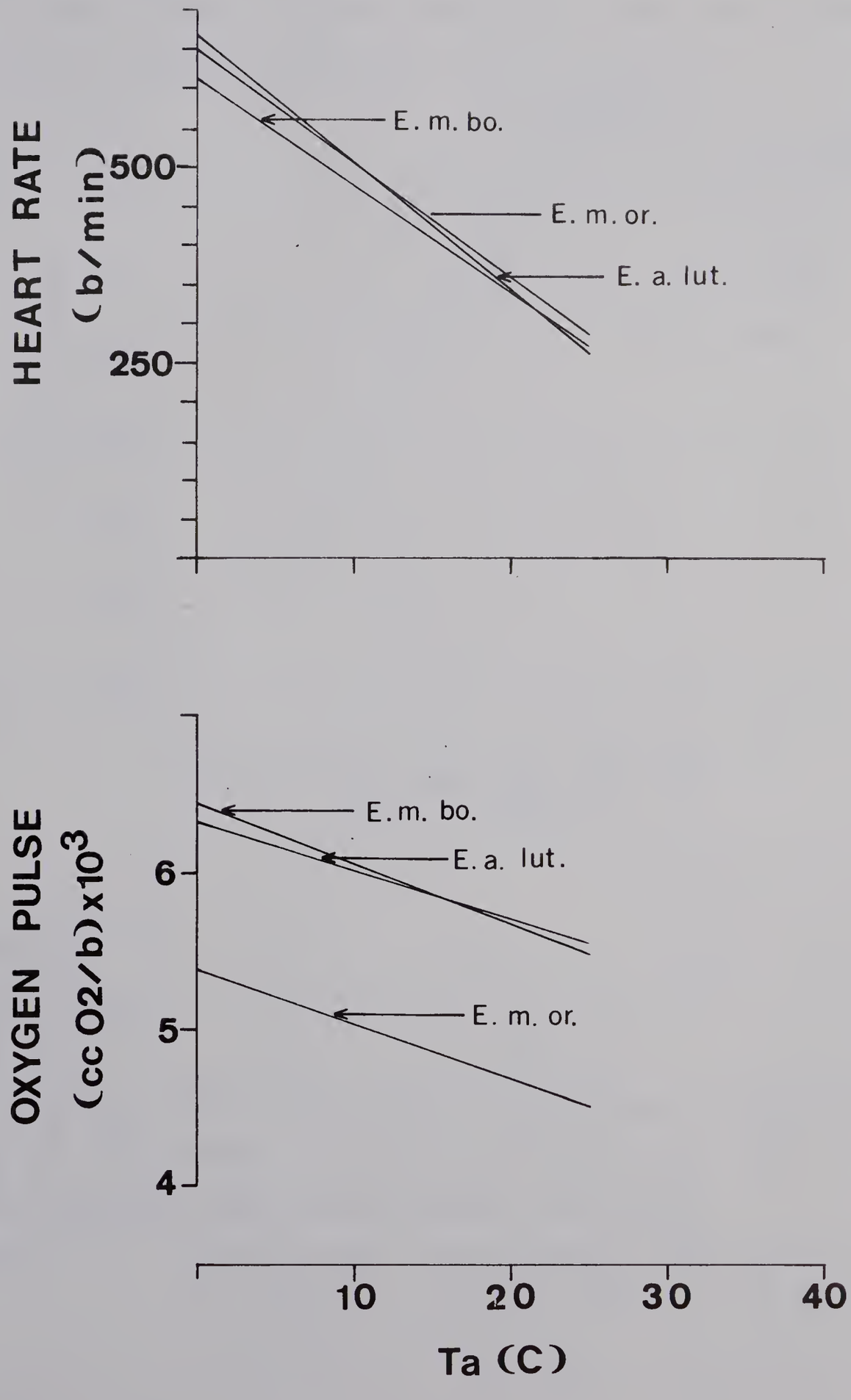

HEART RATE
(b/min)
500
250
E. m. bo.
E. m. or.
E. a. lut.
OXYGEN PULSE
(cc O2/b) x10^3
6
5
4
E. m. bo.
E. a. lut.
E. m. or.
10
20
30
40
Ta (C)

Figure 14A. Oxygen equilibrium curves in percent saturation of haemoglobin as a function of oxygen pressure for Eutamias.

Figure 14B. Measured saturation, PO2 and PCO2 obtained from carotid cannulae with animals under anaesthesia. Center bar indicates mean. End bars give ranges.

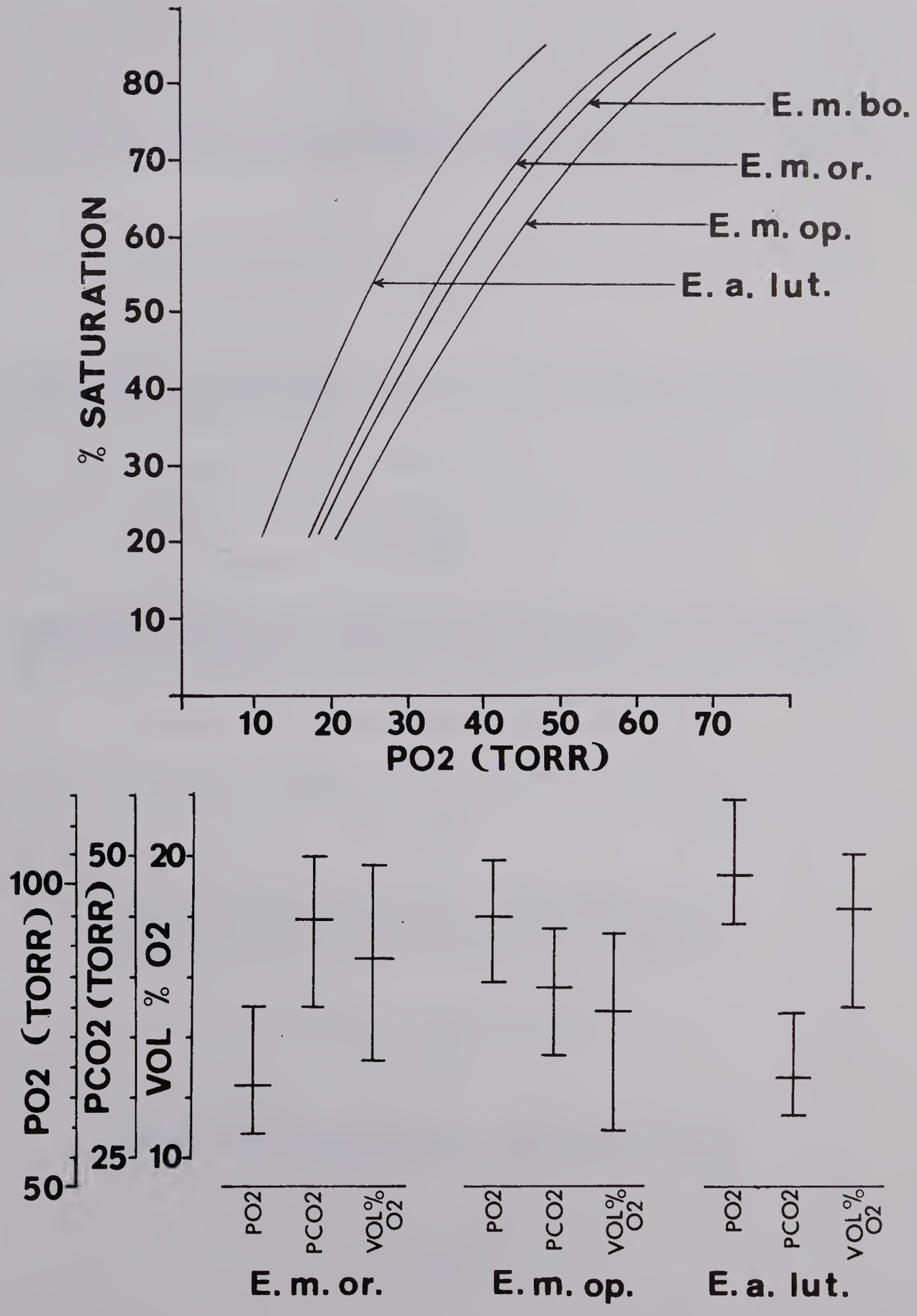
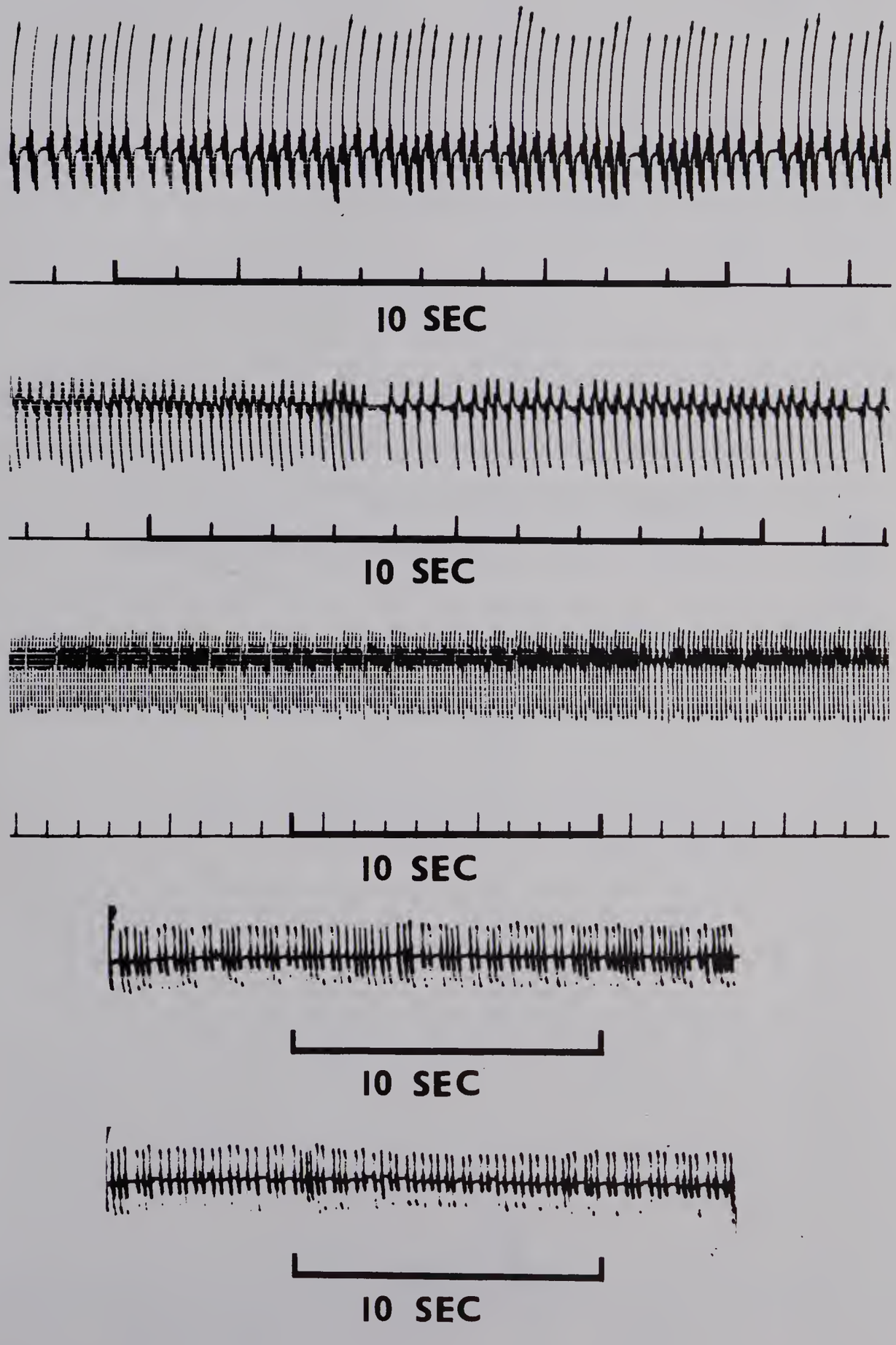

% SATURATION
80
70
60
50
40
30
20
10
10 20 30 40 50 60 70
PO2 (TORR)
E. m. bo.
E. m. or.
E. m. op.
E. a. lut.
PO2 (TORR)
PCO2 (TORR)
VOL % O2
100
50
50
25
20
10
PO2
PCO2
VOL% O2
E. m. or.
E. m. op.
E. a. lut.

Figure 15. Examples of EKG patterns and irregularities. The top three recordings are from animals with direct electrode implants, showing skip beating and rapid alteration of EKG rates suggesting neural control. The bottom two examples were obtained from an animal with an implanted transmitter while in the oxygen analysis in the TNZ.


10 SEC
10 SEC
10 SEC
10 SEC
10 SEC

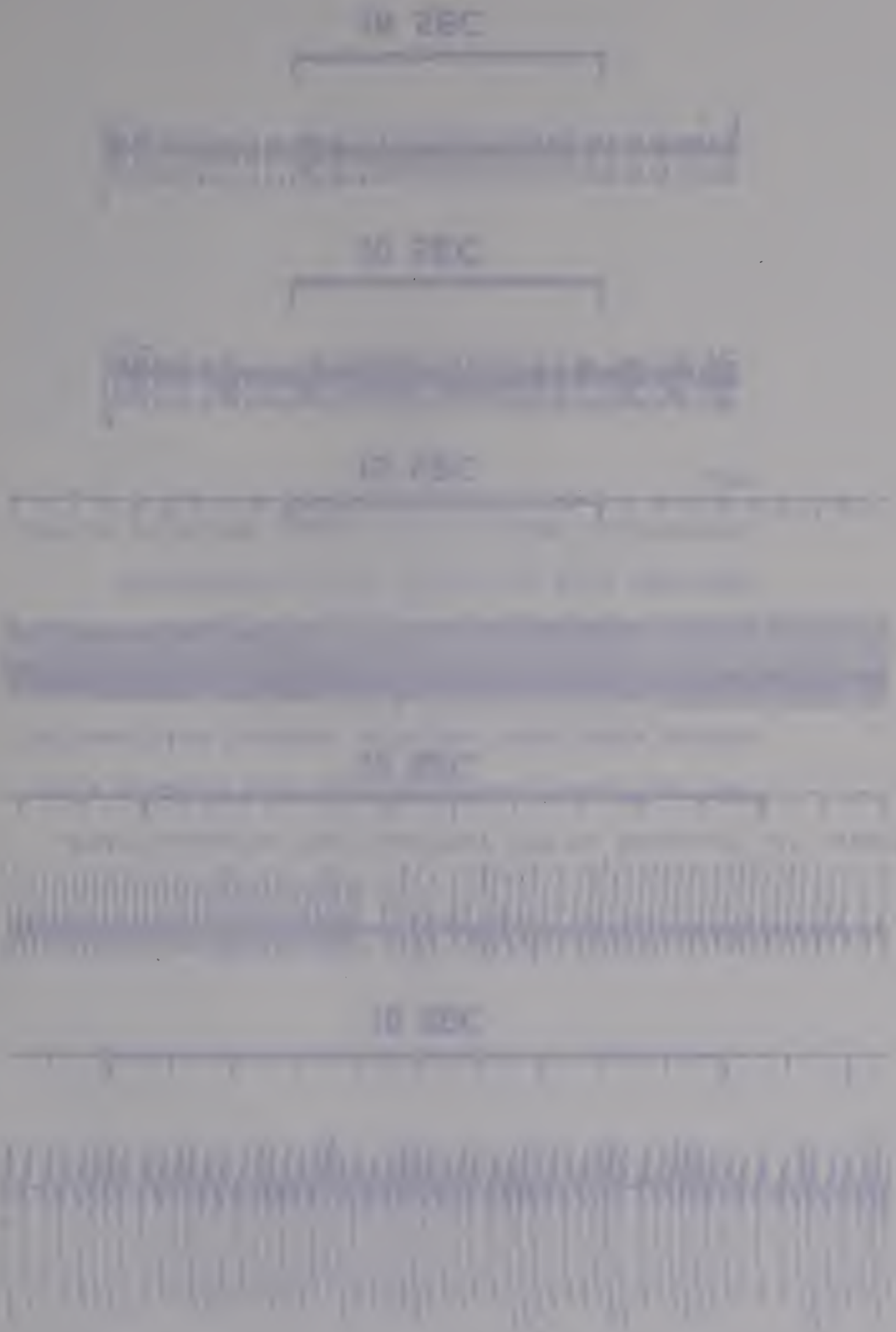


Figure 16. HR of E.a.lut. after operation and in field pen expressed as percent of predicted HR from the E.a.lut. HR versus Ta equation. Black bars indicate "dark" period of the environment.

(■) Ta of the recovery cage

(○) Ta of the rock pile

(·) Ta of the floor of the pen

(△) Ta of the side of the pen on the ledge

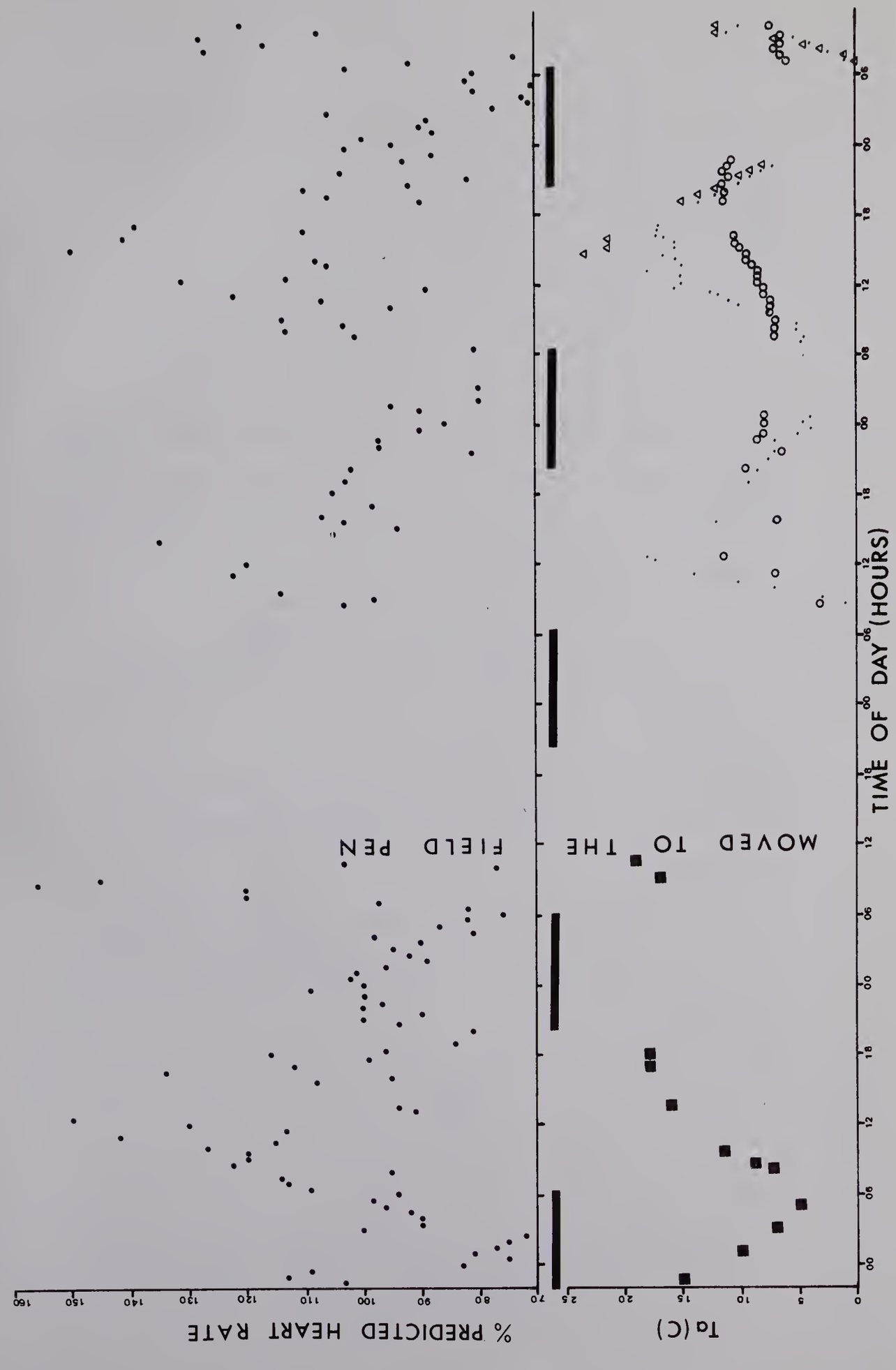

% PREDICTED HEART RATE
Ta (C)
MOVED TO THE FIELD PEN
TIME OF DAY (HOURS)

Figure 17. BHR versus body weight. The equation from Stahl, (1967) (solid line) is $HR = 1419\ W(g)^{-0.25}$. The equation from Wang and Hudson (1971) (dotted line) is $HR = 816\ W(g)^{-0.25}$.

(□) The four subspecies of this study.

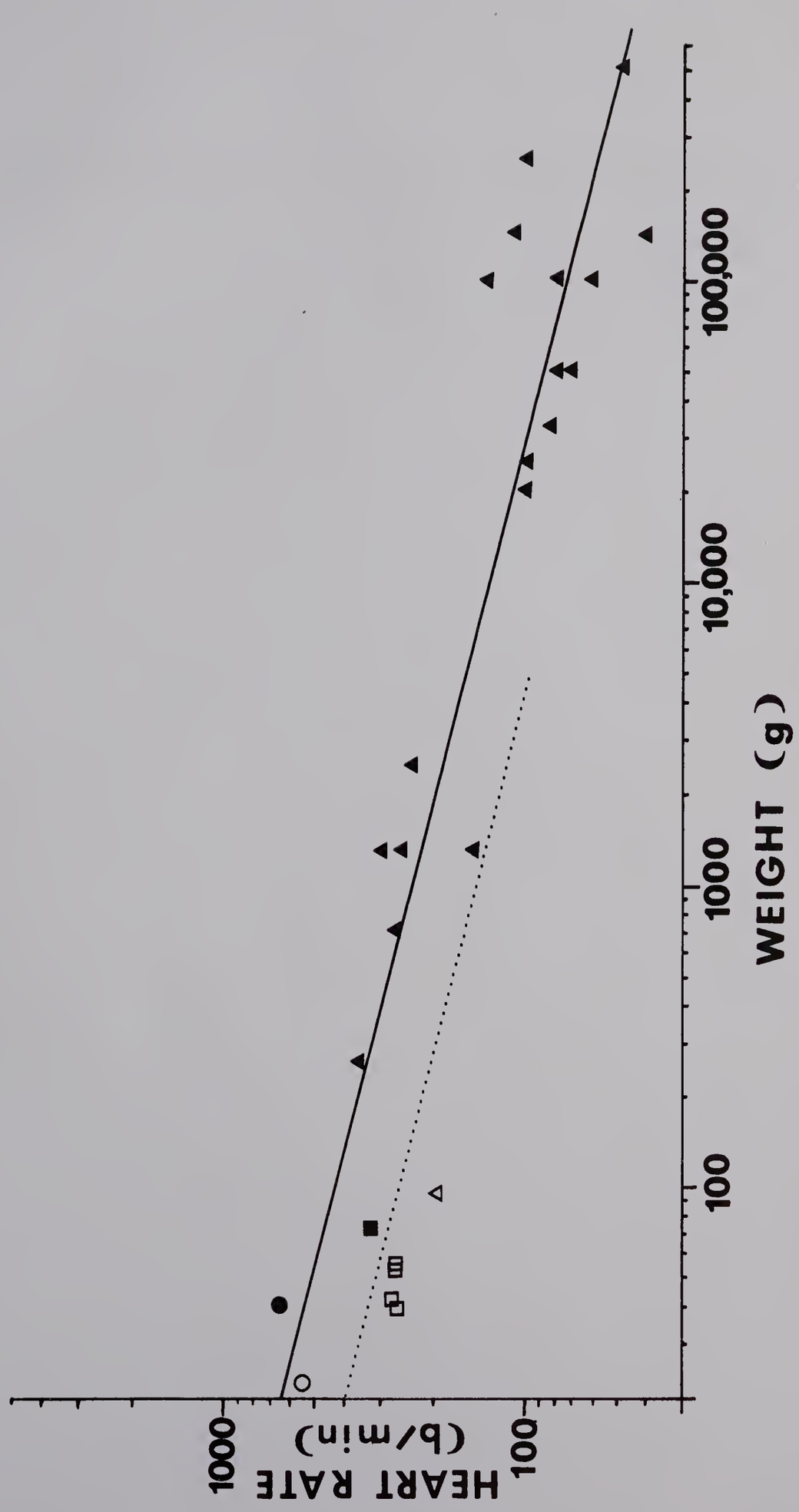
(△) The value of Wang and Hudson (1971) for T. striatus.

(▲) The values from Altman and Dittmer (1966).

(○) The value obtained from Mus mus, Jones - unpublished.

(●) The value reported by Dawe (1953) for E.m..

(■) The value reported by Wunder (1970a) for E. merriami.


HEART RATE (b/min)
1000
100
100
1000
10,000
100,000
WEIGHT (g)